University of Alberta

SYN 1193 as an Alternative to Vancomycin in the Rabbit Model of Peritonitis in Peritoneal Dialysis

by

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©

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Dedication

To my husband, Peter,
our daughter, Jana,
and my parents, Betty and Gordon Lamorie

Abstract

Peritonitis remains a serious complication of PD leading to significant morbidity and mortality. *Staphylococcus aureus* infections have been termed the "Achilles Heel" of PD. Biofilm complicates these infections. Vancomycin is an efficacious and convenient treatment. With development of VRE (1987), VISA (1997) and VRSA (2002) it is imperative that we limit vancomycin use to prevent further resistance. We need alternatives to spare vancomycin use or to use in cases of vancomycin resistance.

We studied SYN 1193 as an alternative to vancomycin to treat MRSA in a rabbit model of PD peritonitis. We used this model to integrate PK/PD parameters and microbiologic response in PD peritonitis.

SYN 1193 20 mg/kg i.p. q12h x 4 days in the first and last of 4 dialysis treatments each day was compared to vancomycin and placebo.

After only 4 days of treatment, SYN 1193 looks promising as an alternative to vancomycin in PD peritonitis. Total concentrations of SYN 1193 providing optimal pharmacodynamic indices of AUC_{24}/MIC ratio, peak/MIC ratio and % t > MIC for treatment of peritonitis were achieved in PDE and plasma. Free concentrations of SYN 1193 in plasma were not optimal for treatment or prevention of resistance. All control rabbits were very ill and euthanised on compassionate grounds. Log_{10} reductions in colony counts in PDE were 4.34 ± 1.09 with SYN 1193 and 3.96 ± 0.68 in the vancomycin group, indicating a >99.99% reduction in bacterial growth in both groups. In

tissue and catheter sites, $\log_{10}$ reductions were 3.85 ± 2.27 with SYN 1193 compared to vancomycin 4.98 ± 1.76. There were no statistically significant differences in the $\log_{10}$ reductions in bacterial growth in PDE or tissue between the two groups $p>0.05$. Time to one $\log_{10}$ reduction in bacterial growth in PDE was the same in both groups.

This rabbit model is excellent for integration of PK/PD with efficacy in PD peritonitis with biofilm infections. Additional studies are required to determine if 14-21 days treatment or optimization of free fraction SYN 1193 would eradicate residual infection. Future studies could include application of MPC and MBEC.

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Table of Contents

1. *Chapter 1*

Introduction

- *1.1. Peritonitis in Peritoneal Dialysis* 1
- *1.2. Treatment of Peritonitis in Peritoneal Dialysis* 2
- *1.3. Pharmacodynamics of Antibacterial Agents* 7
- *1.4. Pharmacodynamics of the Fluoroquinolone Antibiotics* 8
  - *1.4.1. Original Studies of Pharmacodynamics of Fluoroquinolones* 9
  - *1.4.2. Fluoroquinolone Activity Against Streptococcus pneumoniae* 10
  - *1.4.3. Effect of Protein Binding on Pharmacodynamics of Fluoroquinolones* 11
  - *1.4.4. Fluoroquinolone Activity Against Staphylococcus aureus* 13
  - *1.4.5. Pharmacodynamics of Fluoroquinolones Required for Resistance Counterselection* 13
  - *1.4.6. Fluoroquinolone Mutant Prevention Concentration* 15
- *1.5. Vancomycin* 20
  - *1.5.1. Pharmacodynamics of Vancomycin* 20
  - *1.5.2. Effect of Protein Binding on Vancomycin Pharmacodynamics* 25
- *1.6. Biofilm* 28
- *1.7. Rabbit Model* 35
- *1.8. Goal* 36
- *1.9. Hypothesis* 36

2. ***Chapter 2***

Sparfloxacin Assay 37
- *2.1. Chemicals and Materials* 38
- *2.2. Apparatus and Chromatographic Conditions* 38
- *2.3. Preparation of Standard Solutions* 39
- *2.4. Standard Curve Construction* 39

2.5. Sample Preparation 41
2.6. Extraction and Extraction Efficacy 41
2.7. Assay Validation, Accuracy and Precision 42
2.8. Results and Discussion 43
  2.8.1. Chromatograms 43
  2.8.2. Standard Curve Calibration 45
  2.8.3. Accuracy and Precision 46
  2.8.4. PH Dependence of Extraction 50
  2.8.5. Discussion 52
2.9. Conclusions 53

3. Chapter 3
SYN 1193 Assay 54
3.1. Materials and Methods 55
3.2. Apparatus and Chromatographic Conditions 55
3.3. Preparation of Standard Solutions 56
  3.3.1. SYN 1193 Stock Solution 56
  3.3.2. Internal Standard Stock Solution 56
3.4. Sample Preparation 56
3.5. Standard Calibration Curve Construction 57
3.6. Assay Validation 58
3.7. Results and Discussion 59
  3.7.1. Chromatograms 59
  3.7.2. Standard Curve Construction SYN 1193 61
  3.7.3. Precision and Accuracy 66
  3.7.4. Relative Extraction Efficacy SYN 1193 69
3.8. Discussion 70
  3.8.1. Extraction Efficacy 70
  3.8.2. Conclusions 71

4. *Chapter 4*

Comparative Study of SYN 1193, Vancomycin and Controls in the Treatment of Peritonitis in Rabbits Receiving Continuous Ambulatory Peritoneal Dialysis

4.1. Methods 72

- *4.1.1. Rabbit Pre-study* 72
- *4.1.2. Quarantine* 73
- *4.1.3. Preparation for Peritoneal Dialysis* 73
- *4.1.4. Peritoneal Dialysis Catheter Insertion* 73
- *4.1.5. Post-operative Care* 74
- *4.1.6. Isolation Procedures* 75
- *4.1.7. Administration of Test Drug Blood and PDE Sampling* 77

4.2. Results Pre-test Rabbit 78

- *4.2.1. Pre-test Rabbit Receiving SYN 1193* 78
- *4.2.2. Minimum Inhibitory Concentration SYN 1193 against S. aureus MU7056.* 78
- *4.2.3. Plasma and Peritoneal Fluid Concentrations of SYN 1193 in Pre-test Rabbit* 78
- *4.2.4. PDE Samples in SYN 1193 Pre-test Rabbits* 79
- *4.2.5. Peak/MIC Ratios of SYN 1193 Achieved in Plasma and PDE* 80
- *4.2.6. AUC_{24}/MIC Ratios of SYN 1193 Achieved in Plasma and PDE* 81

4.3. Discussion Pre-test Rabbit 81

- *4.3.1. AUC_{24}/MIC Ratios of SYN 1193 Achieved in Plasma and PDE* 83

4.4. Study Animals Receiving SYN 1193, Vancomycin or Placebo 84

- *4.4.1. Inoculation and Induction of Peritonitis – Control Group* 86
- *4.4.2. Drug Administration and Pharmacokinetic Studies* 88
- *4.4.3. Blood Sampling* 88
- *4.4.4. PDE Sampling* 89
- *4.4.5. Urine Collection* 89
- *4.4.6. PDE Samples for Culture* 89
- *4.4.7. Sacrifice and Necropsy* 90

4.4.7.1.Necropsy *90*

4.4.7.2. Samples for Quantitative Bacteriologic Studies and Taxonomic Identification *90*

4.4.7.3. Sample Processing for Quantitative Bacteriologic Studies and Taxonomic Identification *91*

4.4.7.3.1. Tissue Samples for Bacterial Culture *91*

4.4.7.3.2. Sample Processing of Tissues *91*

4.4.7.3.3. Scanning Electron Microscopy *91*

4.4.7.3.4. Evaluation of Results *92*

4.5. Results - Control Animals – Rabbits 1 – 4 *93*

4.5.1. Peritoneal Effluent Cultures for Control Rabbit *94*

4.5.2. Tissue and PD Catheter Sample Culture Results – Control Rabbits 1-4 *96*

4.5.3. Scanning Electron Microscopy of Internal and External Catheter Samples *98*

4.6. Study Test Animals Receiving SYN 1193- Rabbits 9 - 12 *100*

4.6.1. Peritoneal Effluent Cultures Rabbits 9 – 12 Pre- and Post-Treatment with SYN 1193 *100*

4.6.2. Plasma Concentrations and Pharmacokinetic Analysis SYN 1193 in Rabbits 9 – 12 *103*

4.6.3. PDE Concentrations SYN 1193 *109*

4.6.4. Correlation Between SYN 1193 Concentrations in Plasma and PDE *118*

4.6.5. Urinary Excretion SYN 1193 *118*

4.6.6. Peak/MIC and AUC24/MIC Ratios in Plasma for Rabbits Receiving SYN 1193 *120*

4.6.7. Peak/MIC and AUC/MIC Ratios in PDE for Rabbits Receiving SYN 1193 *120*

4.6.8. Tissue and PD Catheter Sample Culture and S.E.M. Results – Rabbits 9 – 12 Treated with SYN 1193 *122*

4.6.9. Log_{10} *Reduction CFU/mL in PDE SYN 1193 Treated Rabbits* *126*

4.6.10. Time to Reduction of Bacterial Growth by 1 Log_{10} with SYN 1193 126
4.6.11. D/P Ratio SYN 1193 128
4.7. Results Rabbits 13A – 16 130
4.7.1. Rabbits 13 – 16 Inoculation 130
4.7.2. Rabbits 13A – 16A Receiving Vancomycin 131
4.7.3. Peritoneal Effluent Cultures Rabbits 13A-16A Pre- and Post-Treatment with Vancomycin 131
4.7.4. Plasma Concentrations Vancomycin in Rabbits 13A-16A 134
4.7.5. PDE Concentrations Vancomycin Rabbits 13A – 16A 140
4.7.6. Culture and S.E.M. Scans of Tissue and Pd Catheter Samples – Vancomycin Group 149
4.7.7. Scanning Electron Microscopy of Internal and External Catheter Samples in Rabbits 13A – 16 A Treated with Vancomycin 149
4.7.8. Log_{10} Reduction CFU/mL in PDE in Vancomycin Treated Patients 153
4.7.9. Time to 1 Log_{10} Reduction PDE Bacterial Growth n Vancomycin Group 153
4.7.10. Efficacy – Treatment of Infection in PDE 155
4.7.11. Efficacy – Treatment of Infection at Tissue / Catheter Sites 159
4.7.11.1. Control Rabbits 159
4.7.11.2. Vancomycin Treated Rabbits 13A-16A 159
4.7.11.3. SYN 1193 Treated Rabbits 9 – 12 160
4.7.11.4. Tissue / Catheter Samples 162
4.8. Discussion Pharmacodynamics – Rabbits Treated with SYN 1193 167
4.8.1. Peak/MIC Ratios SYN 1193 in PDE and Plasma 168
4.8.2. AUC/MIC Ratios SYN 1193 in PDE and Plasma 169
4.8.3. Percent Time Concentrations Greater Than MIC (%t>MIC) 172
4.8.4. Correlation Plasma and PDE Concentrations SYN 1193 179
4.8.5. Pharmacodynamic Targets – Rabbit 13A – 16 A Receiving Vancomycin 180
4.8.6. Peak Vancomycin Concentrations in PDE and Plasma 180

4.8.7. *Vancomycin AUC_{24}/MIC Ratios in PDE and Plasma* 180

4.8.8. *Microbiologic Efficacy* 181

4.8.9. *Survival* 182

4.8.10. *Efficacy in Treatment of Infection in PDE* 182

4.8.11. *Tissue / Catheter Samples* 184

4.8.12. *Tissue / Catheter Results in Rabbits Treated with Vancomycin* 184

4.8.13. *Tissue Catheter Results in Rabbits Treated with SYN 1193* 185

4.8.14. *Induction of Renal Failure in the Rabbit Model* 187

4.8.15. *Future Approaches in the Study of Treatment of Peritonitis and Biofilm in PD* 188

4.9. *Conclusions* 192

4.9.1. *Rabbit Model* 192

4.9.2. *SYN 1193* 193

4.9.3. *Vancomycin* 194

5. ***Chapter 5***

5.1. *Summary and Conclusions* 197

6. ***References*** 200

List of Tables

1. Chapter 1 – Introduction

1.1 MICs (μg/mL) SYN 1193 against 16 Ciprofloxacin Sensitive and Ciprofloxacin- Resistant Strains 6

1.2 Antibacterial Activity of SYN 1193, Ciprofloxacin and Vancomycin against MSSA, Ciprofloxacin moderately-resistant MRSA and Ciprofloxacin-highly-resistant MRSA 6

1.3 Comparative In Vitro Minimum Inhibitory Concentrations of Newer Quinolones 7

1.4 Compilation of studies done to elucidate the pharmacodynamics of vancomycin. 27

2. Chapter 2 – Sparfloxacin Assay

2.1 Sparfloxacin Standard Solutions for Construction of Calibration Curve 40

2.2 Intraday Coefficient of Variation of Sparfloxacin Assay 47

2.3 Interday Coefficient of Variation and Accuracy 48

2.4 Accuracy and Precision of Sparfloxacin Assay 49

2.5 Extraction efficacy in ethyl acetate at varying pH levels. 50

Chapter 3 – SYN 1193 Assay

3.1 SYN 1193 Calibration Curve Construction 58

3.2 Standard Curve Construction 61

3.3 Intra-day Coefficient of Variation and % Error of SYN 1193 67

3.4 SYN 1193 Accuracy 68

3.5 Relative Extraction Efficacy 69

4. Chapter 4

Comparative Study of SYN 1193, Vancomycin and Controls in the Treatment of Peritonitis in Rabbits Receiving Continuous Ambulatory Peritoneal Dialysis

4.1 Plasma Concentrations of SYN 1193 for Pre-test Rabbit 79

4.2 PDE concentrations of SYN 1193 in Pre-test Rabbit 80

4.3 Peak concentrations of SYN 1193 in plasma and PDE and Peak/MIC ratios 80

4.4 AUC SYN 1193 and AUC_{24}/MIC ratio in Plasma and PDE 81

4.5 AUC24/MIC ratio SYN 1193 in plasma and PDE 83

4.6 Cultures PDE Control Rabbits 95

4.7 Tissue and PD Catheter Sample Culture Results – Control Rabbits 97

4.8 Electron Microscopy and Cultures (CFU/g/mL) of Internal and External Catheters 99

4.9 PDE Cultures Pre and Post Treatment with SYN 1193 102

4.10 Plasma Concentrations SYN 1193 Rabbits 9 – 11. 104

4.11 PDE Concentrations SYN 1193 Rabbits 9 -11 110

4.12 Urinary Excretion SYN 1193 119

4.13 Peak/MIC and AUC_{24}/MIC Ratios in Plasma for Rabbits Receiving SYN 1193 121

4.14 Peak/MIC and AUC24/MIC Ratios in PDE for Rabbits Receiving SYN 1193 122

4.15 Tissue/Catheter Sample Culture Results Post Treatment with SYN 1193 124

4.16 Scanning Electron Microscopy and bacterial growth (CFU/g/mL) of Internal and External Catheter Samples in Rabbits Treated with SYN 1193 125

4.17 Log_{10} Reduction of Bacterial Growth in PDE of SYN 1193 Treated Rabbits 127

4.18 D/P Ratios SYN 1193 – Rabbits 9 -11 129

4.19 PDE Cultures Pre and Post Treatment with Vancomycin 133

4.20 Plasma Concentrations of Vancomycin Rabbits 13A – 16A 135

4.21 PDE Vancomycin Concentrations 141

4.22 Plasma Vancomycin 24 hr AUC, AUC_{24}*/MIC ratio, t>MIC and extrapolated t > MIC.* 146

4.23 PDE Vancomycin 24 hr AUC, AUC_{24}*/MIC ratio, t>MIC and Extrapolated t >MIC.* 146

4.24 Plasma and PDE Concentrations of Vancomycin and D/P Ratios. 147

4.25 Tissue / Catheter Sample Culture Results in Vancomycin Treatment Group 151

4.26 Scanning Electron Microscopy of Internal and External Catheter Samples in Rabbits 13A – 16A Treated with Vancomycin 152

4.27 Log_{10} *Reduction of CFU/mL in PDE of Vancomycin Treated Rabbits* 154

4.28 Time to One Log_{10} *CFU/g/mL Reduction in PDE Bacterial Growth in Vancomycin Group* 157

4.29 Time to One Log_{10} *Reduction with SYN 1193 Treatment* 158

4.30 Colony counts (CFU/g/mL) of tissues and Log_{10} *reduction in CFU/g/mL post-treatment with SYN 1193 and vancomycin relative to controls.* 164

4.31 Comparison Residual Infection in Tissue/Catheter/ Sites in Vancomycin, SYN 1193 and Controls 165

List of Figures

1. *Chapter 1 Introduction*

1.1. *AUC24/MIC total and free drug concentrations associated with effective treatment in animal models* 12

1.2. *Mutant Selection Concentrations* 16

1.3. *Mutant Selection Window* 18

1.4. *Process of Biofilm Formation* 30

1.5. *Detachment and Dispersal of Planktonic and Sessile Forms of Bacterial Cells from Biofilm* 30

2. *Development of Sparfloxacin Assay*

2.1. *Chemical Structure Sparfloxacin* 37

2.2. *Representative Chromatograms of blank rabbit plasma and plasma spiked with ciprofloxacin and sparfloxacin.* 44

2.3. *Sparfloxacin Standard Curve* 45

2.4. *pH Dependence of Extraction of Sparfloxacin* 51

3. *Development of SYN 1193 Assay*

3.1. *Chemical Structure SYN 1193* 54

3.2. *Representative Chromatograms* 60

3.3. *Representative Standard Curve SYN 1193 in Plasma* 63

3.4. *Representative Standard Curve SYN 1193 in Dianeal 1.5%* 64

3.5. *Representative Standard Curve SYN 1193 in PDE* 65

4. Chapter 4 Comparative Study of SYN 1193, Vancomycin and Placebo in the Treatment of Peritonitis in Rabbits receiving Continuous Ambulatory Peritoneal Dialysis

4.1. *Rabbit Model* 75

4.2. *Dialysis Schedule* 78

4.3. *Research Protocol* 86

4.4. *Plasma Concentrations SYN 1193 Rabbit 9* 105

4.5. *Plasma Concentrations SYN 1193 Rabbit 10* 106

4.6. *Plasma Concentrations SYN 1193 Rabbit 11* 107

4.7. *Plasma Concentrations SYN 1193 Rabbit 12* 108

4.8. *PDE Concentrations SYN 1193 Rabbit 9* 111

4.9. *PDE Concentrations SYN 1193 Rabbit 10* 112

4.10. *PDE Concentrations SYN 1193 Rabbit 11* 113

4.11. *Plasma and PDE Concentrations SYN 1193 Rabbit 9* 114

4.12. *Plasma and PDE Concentrations SYN 1193 Rabbit 10* 115

4.13. *Plasma and PDE Concentrations SYN 1193 Rabbit 11* 116

4.14. *Plasma Concentrations SYN 1193 Rabbit 12* 117

4.15. *Correlation Between PDE and Plasma Concentrations SYN 1993* 118

4.16. *Comparison D/P Ratios SYN 1193 Rabbits 9, 10, and 11* 128

4.17. *Vancomycin Plasma Concentrations Rabbit 13A* 136

4.18. *Vancomycin Plasma Concentrations Rabbit 14A* 137

4.19. *Vancomycin Plasma Concentrations Rabbit 15A* 138

4.20. *Vancomycin Plasma Concentrations Rabbit 16A* 139

4.21. *Vancomycin PDE Concentrations Rabbit 13A* 142

4.22. *Vancomycin PDE Concentrations Rabbit 14A* 143

4.23. *Vancomycin PDE Concentrations Rabbit 15A* 144

4.24. *Vancomycin Plasma Concentrations Rabbit 16A* 145

4.25. *D/P Ratio Vancomycin* 148

4.26. *Log_{10} Reduction of CFU/mL in PDE in SYN 1193 and Vancomycin Treated Rabbits* 156

4.27. *Representative S.E.M. of Biofilm on the External Catheter* 160

4.28. *Representative S.E.M. of Biofilm on the Internal Catheter* 161

4.29. *Representative S.E.M. of Biofilm on the Dacron Cuff.* 162

4.30. *Mean log_{10} Reduction in Bacterial Growth (CFU/g/mL) in all Tissue and Catheter Samples in SYN 1193 and Vancomycin Treated Groups.* 166

4.31. *Total and Free Plasma and PDE Concentrations SYN 1193 Rabbit 9* 176

4.32. *Total and Free Plasma and PDE Concentrations SYN 1193 Rabbit 10* 177

4.33. *Total and Free Plasma and PDE Concentrations SYN 1193 Rabbit 11* 178

List of Abbreviations

ACN	acetonitrile
APD	automated peritoneal dialysis
AUC	area under the curve
MIC	minimum inhibitory concentration
AUC/MIC	area under the concentration-time curve to minimum inhibitory concentration ratio
AUC_{24}/MIC	area under the 24 hour concentration-time curve to minimum inhibitory concentration ratio
AUIC	area under the inhibitory curve
b.i.d.	twice a day
CAPD	Continuous ambulatory peritoneal dialysis
CBD	Calgary Biofilm Device
CDC	Center for Disease Control in Atlanta
Cip	Ciprofloxacin
Cmax	Maximum concentration
Conc	Concentration
CV	coefficient of variation
D/P	Dialysate /Peritoneal Fluid Ratio
D/P_{cr}	Dialysate Creatinine/Peritoneal Fluid Creatinine Ratio
ER	extraction ratio
EPS	extracellular polymeric substances

$f\mathrm{AUC}_{24}/\mathrm{MIC}$	area under the 24 hour free concentration-time curve to minimum inhibitory concentration ratio
f peak/MIC ratio	free peak concentration to minimum inhibitory concentration
f %t>MIC	% time free concentration greater than minimum inhibitory concentration
GISA	glycopeptide-resistant *Staphylococcus aureus*
HPLC	High-Performance Liquid Chromatography
hr	hour(s)
i.p.	intraperitoneal
ISPD	International Society of Peritoneal Dialysis
i.m.	intramuscular
IS	Internal standard
I.V.	intravenously
K. pneumoniae	*Klebsiella pneumoniae*
Kg	kilogram
MIC	minimum inhibitory concentration
μL	microlitre
μg	microgram
μg/mL	microgram/mL
mL	milliliter
mg	milligram
mg/L	milligram/L
NCCLS	National Committee for Clinical Laboratory Standards
HD	hemodialysis

L	litre
MBC	minimum bactericidal concentration
MBEC	minimum biofilm eradication concentration
MPC	mutant prevention concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
MSW	mutant selection window
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
PAE	post-antibiotic effect
peak/MIC ratio	peak concentration to minimum inhibitory concentration
PBS	phosphate buffered saline
PK/PD	pharmacokinetic/pharmacodynamic
P. aeruginosa	*Pseudomonas aeruginosa*
PD	Peritoneal Dialysis
PDE	Peritoneal Dialysis Effluent
PDF	Peritoneal Dialysis Fluid
PET	Peritoneum Equilibrium Test
PK/PD	Pharmacokinetic/Pharmacodynamic
q.i.d.	four times a day
q.s.	Make up to final volume
q12h	every 12 hours
R	resistant
RRF	Residual Renal Function

S	Sensitive
S. aureus	*Staphylococcus aureus*
SBA	serum bactericidal concentration
S.D.	Standard Deviation
S.E.	Standard Error
S. epidermidis	Staphylococcus epidermidis
S.E.M.	scanning electron microscopy
t>MIC	time greater than MIC
%t>MIC	% time greater than MIC
$t_{1/2}$	Elimination half-life
TEA	Triethylamine
t.i.d.	three times a day
T_{max}	Time of maximum concentration
TEA	Triethylamine
UV	ultraviolet
VRE	Vancomycin resistant enterococci
VISA	vancomycin intermediate-resistant *Staphylococcus aureus*
VRSA	vancomycin-resistant *Staphylococcus aureus*
Vd	volume of distribution
o.d.	daily

Chapter 1

Introduction

Infective peritonitis is the major complication of continuous ambulatory peritoneal dialysis (CAPD) (1, 2) and is the leading cause of technique failure among patients on CAPD therapy (3). Peritonitis occurs at a rate of approximately 0.3 – 0.5 cases per patient year (4) contributing to hospitalization, technique failure and mortality. Hospitalization rates in adult PD patients due to peritonitis, reported by Fried et al., were second only to cardiac disease accounting for 13.5% and 14.6% of admissions. Overall, there were 1.6 admissions and 13 hospital days per patient year (5). Death, attributable to peritonitis, occurs in up to 15.8% of patients (6). *S. aureus, P. aeruginosa,* and fungal pathogens are known to cause aggressive forms of peritonitis with high treatment failure rates.

Infection develops in 60-70% of CAPD patients in the first year. Recurrent infections develop in 20-30% (7). Peritonitis is most commonly caused by gram-positive bacteria. A Canadian study by Zelenitsky et al., from 1991 – 1998, reported that 27.8% of culture positive cases were due to *Staphylococcus epidermidis (S. epidermidis)* and 19.3% *Staphylococcus aureus (S. aureus). Pseudomonas aeruginosa (P. aeruginosa) (7.1%), Escherichia coli (E. coli) (6.8%),* and *Klebsiella pneumoniae (K. pneumoniae) (5.2%)* were the most common gram-negative organisms identified (8). Anaerobes (2.6%), fungi (2.6%) and mycobacteria may also contribute to the development of peritonitis. Over the period of time of this study, with improved disconnect systems and use of the twin-bag system, the rate of peritonitis decreased from 1.37 to 0.55 episodes/patient-year. The rate of gram-positive peritonitis decreased from 0.75 to 0.28 episodes/patient-year while the rate of gram-negative peritonitis remained constant. In this study, dramatic increases in antibiotic resistance in *S. epidermidis* were seen over this period of time. Resistance to methicillin increased from 18.9 to 73.9% while resistance to ciprofloxacin increased from 5.4 to 47.8%.

Peritonitis remains a significant problem in peritoneal dialysis, indeed peritonitis, in particular peritonitis due to *S. aureus*, has been referred to as the "Achilles Heel" of peritoneal dialysis (4, 9).

Treatment of Peritonitis in Peritoneal Dialysis

The treatment of peritonitis in peritoneal dialysis has changed over the years, in large part due to the growing problems with antibiotic resistance. In 1987, The International Society of Peritoneal Dialysis (ISPD) established an ad hoc committee to provide guidelines for the optimal treatment of peritonitis in peritoneal dialysis (10). This committee recommended either vancomycin or a first generation cephalosporin as empiric therapy for peritonitis caused by gram-positive pathogens in 1987 and again in1989 (10, 11). In 1993, the committee changed its recommendation to vancomycin as the first-line agent. Vancomycin was effective and very convenient requiring infrequent dosing in PD patients due to its poor dialysability and has been used more frequently than truly required due to this convenience.

Increasing use of vancomycin world-wide has lead to the development of resistance. Vancomycin-resistant enterococci (VRE) with resistance to multiple antibiotics were first reported in the United Kingdom and France in 1987 (12). By 1993, 14% of enterococcal isolates from patients in intensive units in the United States were vancomycin resistant, a 20-fold increase since 1987. In addition to the development of vancomycin resistance, eighty-eight percent of these isolates were also multiply resistant to ß-lactams, aminoglycosides, fluoroquinolones, tetracycline, chloramphenicol, and teichoplanin (12), leaving no good alternatives for treatment. Thirty-six percent of patients with primary blood stream infections with VRE died (12).There were concerns throughout the scientific and lay community that we had come to the "End of the Antibiotic Era." Concerns about increasing resistance to vancomycin prompted the Center for Disease Control in Atlanta to develop guidelines, published in 1995, to limit the use of vancomycin (13).

In response to concerns of increasing vancomycin resistance, the International Society of Peritoneal Dialysis ad hoc committee published a new set of guidelines in 1996 recommending cefazolin, rather than vancomycin, as the first line agent for the treatment of gram-positive infections, and a combination of cefazolin and an aminoglycoside for empiric therapy. Since then, there have been increasing problems with vancomycin resistance. In 1997, the first case of *S. aureus* with intermediate resistance to vancomycin (VISA) was reported in Japan with several reports in the United States soon to follow (14). Although initially, several risk factors were correlated with the development if VISA, a study of *S. aureus* with reduced susceptibility to vancomycin and VISA in the United States from 1997 - 2001 revealed that the only independent risk factors for the development of *S. aureus* with reduced susceptibility to vancomycin were an antecedent infection with MRSA and vancomycin use in the preceding 3 months (15).

The I.S.P.D. peritonitis treatment guideline update, published in 2000, recognizing the increasing concerns about vancomycin resistance, recommended administration of a first generation cephalosporin (cefazolin or cephalothin – 1 g daily in the long dwell) in combination with ceftazidime for empiric treatment in PD patients with peritonitis.

In June 2002, the inevitable came to pass, and the first case of *S. aureus* fully resistant to vancomycin (VRSA) was reported in Michigan in a diabetic, hemodialysis patient receiving > 2 months vancomycin therapy for the treatment of leg ulcers and an infected arteriovenous graft (16). By September 2002, a second case was reported in Pennsylvania (17). Fortunately some new alternative drugs such as quinupristin/dalfopristin and linezolid with activity against VRSA and VRE have come on the market. However, it is quite alarming that the first cases of resistance to linezolid have already been reported (18-20). A paper by Golper et al. in 2000, referring to increasing problems with resistance used the analogy of vancomycin as the knight in shining armour that has been eaten by the dragon (21). It is clear now, with the development of further resistance to vancomycin and alternate choices, that not only has Sir Lancelot been slain, but, the dragon is now winning its fight against the other Knights of the Round Table. In addition to increasing problems with the development of resistance, concerns have been raised in the medical community that a dwindling number of pharmaceutical firms are investing in the development of new antimicrobial agents (22) (23).

The newest I.S.P.D. recommendations for the treatment of peritonitis, published in March 2005, state that empiric therapy should be centre-specific based on resistance patterns. Current recommendations state that gram-positive organisms may be covered by vancomycin or a cephalosporin and gram-negative organisms by a third generation cephalosporin or an aminoglycoside, ceftazidime, cefepime or carbapenem (1). The very mention of vancomycin -"our knight in shining armour" against gram-positive infections, an aminoglycoside, cefepime (a fourth generation cephalosporin) and a carbapenem all as choices for first line agents in the treatment of peritonitis is a true testament to the difficulties encountered in treating organisms such as *P. aeruginosa* and *S. aureus* in peritonitis and the tremendous problems encountered with antibiotic resistance that have developed with years of antibiotic use and overuse.

It is clear that we need to attack the issues of antibiotic resistance. It is also clear that we need alternatives to vancomycin. In a particular, we need alternatives for the treatment of methicillin-resistant *S. aureus,* either as choices for treatment of strains resistant to vancomycin, or alternatives to vancomycin to spare the use of vancomycin and limit vancomycin resistance. SYN 1193 is an investigational 8-methoxy-fluoroquinolone discovered by Synphar Laboratories, Edmonton, Alberta. SYN 1193 has enhanced activity against gram-positive bacteria including MSSA, MRSA and ciprofloxacin-resistant MRSA (24). In addition, SYN 1193 has good gram-negative coverage. As such, this drug may serve as an alternative to vancomycin in the treatment of MRSA peritonitis. SYN 1193 has not been studied in peritonitis in CAPD. In vitro studies by Dasgupta et al. of its activity against MRSA biofilm coated silicone catheters look very promising (25). The in vitro activity of SYN 1193 against MRSA and ciprofloxacin-resistant MRSA as published by Kitzis et al. are shown in **Table 1.1** (26). In these studies, SYN 1193 was found to be 16 – 64 times more active than older fluoroquinolones against all streptococci and ciprofloxacin-resistant *S. aureus (26).* Results of studies of antibacterial activity of SYN 1193 by Synphar comparing the activity of SYN 1193 to ciprofloxacin and vancomycin against MSSA, ciprofloxacin-moderately-resistant MRSA, and ciprofloxacin-highly-resistant MRSA are shown in **Table 1.2** (24). Comparative in vitro MIC data for the newer fluoroquinolones against *S. aureus,* MRSA and ciprofloxacin-resistant *S. aureus* as compiled by O'Donnell et al. are presented in **Table 1.3** (27). Overall, SYN 1193 appears to be more active against *S. aureus, and* MRSA than the newer fluoroquinolones levofloxacin, gatifloxacin, moxifloxacin, and gemifloxacin when comparing values in **Tables 1.2 and 1.3**. Incomplete data was available for the investigational desfluoroquinolone garenoxacin (27).

Table 1.1. MICs (μg/mL) SYN 1193 against 16 Ciprofloxacin-Sensitive and Ciprofloxacin-resistant *S. aureus (26).*

	Number of strains at each indicated MIC (μg/mL)								
	0.007	**0.0015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**
Cip S	14	2							
Cip R					1	30	6	8	1

Abbreviations: Cip R = ciprofloxacin resistant, Cip S = ciprofloxacin sensitive

Table 1.2 Antibacterial activity of SYN 1193, Ciprofloxacin and Vancomycin against MSSA, ciprofloxacin moderately resistant (MIC 12.5-50) MRSA, and Ciprofloxacin-highly resistant (MIC ≥ 100) MRSA.

Organism	**MIC Range**		**MIC (μg/mL)**	
(Number) Drug	**(μg/mL)**	**50%**	**80%**	**90%**
MSSA (50 strains)				
SYN 1193	≤ 0.0125 – 0.025	0.025	0.025	0.025
Ciprofloxacin	0.2 -6.25	0.78	3.13	3.13
Vancomycin	0.39 – 1.56	0.78	0.78	0.78
Ciprofloxacin-Moderately-Resistant (MIC 12.5-50) MRSA (22 strains)				
SYN 1193	0.2 – 0.78	0.39	0.78	0.78
Ciprofloxacin	12.5 – 50	25	50	50
Vancomycin	0.39 – 1.56	0.78	0.78	0.78
Ciprofloxacin-Highly-Resistant (MIC ≥100) MRSA (37 strains)				
SYN 1193	0.39 – 3.13	0.78	1.56	1.56
Ciprofloxacin	100 - >100	100	>100	>100
Vancomycin	0.39 – 1.56	0.78	0.78	0.78

Table 1.3 Comparative In Vitro Minimum Inhibitory Concentrations Of Newer Quinolones (MIC_{90})

Organism	***Levofloxacin***	***Gatifloxacin***	***Moxifloxacin***	***Gemifloxacin***	***Garenoxacin***
S. aureus	*0.25*	*0.10 – 0.13*	*0.06*	*0.03*	*0.03*
MRSA	*16*	*0.20 - 16*	*4*	*4*	–
CRSA	*8*	*6.25*	*2*	*8*	–

Pharmacodynamics of Antibacterial Agents

Pharmacokinetics refers to the study of the time course of a drug (the antibacterial agent) in the body. With antibacterial agents pharmacodynamics relates the fluctuating antibiotic concentrations after administration (the pharmacokinetics) to the antimicrobial effect and/or adverse effects. The ultimate target in the treatment of infectious diseases is the bacterial cell. In the laboratory, antibiotic activity can be assessed by measurement of the minimum inhibitory concentration (MIC) of an antibiotic against a specific bacterial strain by using serially diluted concentrations of the antibiotic in a suitable growth medium with a standard inoculum of the bacterial strain at pH 7.2 (28). During this test, the concentration of the antibiotic remains constant over the test interval. When antibiotics are given to a patient the concentrations of the antibiotic may vary tremendously over the dosing interval from peak to trough. These variations in antibiotic concentration may influence the effect of the antibiotic in killing the bacterial cell. An in vitro model has been developed to simulate various patterns of antibiotic concentrations over time and determine the effect of these variations using time-kill studies. Further studies have been done in neutropenic and non-neutropenic animal models as well as humans to determine the pharmacodynamic parameters that correlate with bacterial killing or efficacy of antimicrobial agents in the treatment of infectious diseases (29-33).

Antibiotics fall into three groups based on their pharmacodynamic properties. The first group includes antibiotics that induce concentration-dependent killing and produce prolonged persistent effects. The classic examples of concentration-dependent antibiotics are the aminoglycosides and fluoroquinolones. Goals of therapy with these drugs are to enhance peak concentrations and antibiotic exposure relative to the MIC of the infecting organism as measured by the peak/MIC ratio and area under the plasma concentration time curve AUC_{24}/MIC ratio. The second group exhibit time-dependent killing with minimal or no post-antibiotic effects. The goal of treatment with this group of antibiotics is to optimize the period of time the concentration of the antibiotic remains above the MIC of the organism throughout the dosing interval (t>MIC or %t>MIC). A %t>MIC of 40-50% frequently correlates with efficacy. The classic example of time-dependent antibiotics are the ß-lactam antibiotics. The third group includes drugs that display time-dependent killing but have more prolonged persistent effects. The goal of treatment with these drugs is to provide adequate exposure to the drug. With this group an adequate AUC_{24}/MIC ratio is the most important target to achieve. The azalides and lincosamides are examples of antibiotics that fit into this category (28, 30).

Pharmacodynamics of the Fluoroquinolone Antibiotics

The fluoroquinolone antibiotics exhibit concentration dependent killing, consequently, bacterial killing occurs most rapidly when drug concentrations are much higher than the MIC of the organism (32). Fluoroquinolone antibiotics also have a moderate to prolonged post-antibiotic effect which is concentration dependent (34) lasting 1.5 – 2.5 hr for gram-positive and gram-negative infections (35). Post-antibiotic effects of the newer quinolones have been reported to range from 1-6 hours (27).

The understanding of the pharmacodynamic parameters associated with successful treatment and development of resistance with the fluoroquinolone group of antibiotics has steadily evolved over the past 15 years with many new findings published in the last few years.

Original Studies of Pharmacodynamics of Fluoroquinolones

Animal studies by Leggett and Craig et al. in 1990 on the pharmacodynamics of the ciprofloxacin in neutropenic murine thigh model infected with gram-negative organisms demonstrated that the pharmacodynamic parameter AUC_{24}/MIC correlated best with efficacy. An AUC_{24}/MIC ratio of 50 was associated with a bacteriostatic effect while an AUC_{24}/MIC ratio of ≥ 100 was associated with a 1 to 2 $\log_{10}$ (90%-99%) reduction in bacterial growth. AUC_{24}/MIC ratios of 50 and 100 were associated with an animal survival rate of 50% and 90% respectively (34).

The first human studies by Peloquin et al. were in patients seriously ill with pneumonia in intensive care receiving ciprofloxacin (36). Time of exposure to ciprofloxacin was found to be important for clinical success. In addition, a low peak/MIC ratio of < 10 was associated with the development of resistance. Through further analysis of this group of patients, and the addition of 24 more patients, Forrest and Schentag et al. determined that the most important predictor of clinical and microbiologic cure was total AUC_{24}/MIC ratio (reported as AUIC) of ≥125. At an AUIC < 125, the probabilities of clinical and microbiologic cure were only 42 and 26% respectively while at an AUIC ≥125, these probabilities were 80 and 82%. The AUIC also predicted time to eradication of pathogen. At an AUIC < 125 the median time to eradication was > 32 days, at an AUIC of 125-250 time to eradication was 6.6 days and at an AUIC > 250 time to eradication was only1.9 days (37).

significantly better survival in the neutropenic rat model of *P. aeruginosa* infection, however, if the peak /MIC ratio was < 10 the AUC_{24}/MIC ratio appeared most closely linked to outcome (39). In a study of patients with skin, respiratory or urinary tract infections treated with levofloxacin, Preston et al. found that clinical outcome was best predicted by the site of infection and a peak/MIC ratio of >12.2, however, clinical outcome was also highly correlated with AUC_{24}MIC ratio since peak and AUC are interrelated (40).

It became commonly accepted that for all fluoroquinolone antibiotics, the pharmacodynamic parameters that one must attain to ensure effective therapy were an AUC_{24}/MIC of 100 – 125 (41) and a peak /MIC ratio of >10 as reported in a comprehensive review of the pharmacodynamics of the fluoroquinolones by Turnidge in 1999 (42).

Fluoroquinolone Activity Against Streptococcus pneumoniae

With increasing resistance of *S. pneumoniae* to traditional agents and development of newer quinolones with greater activity against gram-positive organisms, work was done to elucidate the pharmacodynamics of these agents against gram-positive organisms such as *S. pneumoniae*.

Using an in vitro system to study the eradication of *S. pneumoniae* with ciprofloxacin, levofloxacin, trovafloxacin and ofloxacin, Lister et al. reported that AUC_{24}/MIC ratios of 32 – 64 were associated with eradication of *S. pneumoniae* (43). Lacy et al., using an in vitro model, demonstrated that AUC_{24}/MIC ratios of levofloxacin and ciprofloxacin of 30 - 50 or greater were sufficient to eradicate *S. pneumoniae* regardless of the MIC (44). Hershberger and Ryback studied the pharmacodynamics of six fluoroquinolones in an in vitro fibrin clot model infected with PRSP. Although there was no clear association between pharmacodynamic parameters and bacterial killing, an AUC_{24}/MIC ≤40 or %t>MIC ≤55 was associated with decreased killing and significant bacterial regrowth (45). Lister et al. studied the pharmacodynamics of gatifloxacin and levofloxacin against *S. pneumoniae* in an in vitro study designed specifically to determine the effect of varying the AUC_{24}/MIC ratio while holding the peak/MIC ratio constant at 2 to 3 fold above the MIC. This study determined that continued killing and eradication were observed only when gatifloxacin and levofloxacin AUC_{24}/MIC ratios were 27 – 48 and 30 – 38 respectively (46). With this work, it became accepted that the pharmacodynamic targets for treatment were different with gram-positive and gram-negative infections – in other words " One size does not fit all." (47)

Effect of Protein Binding on Pharmacodynamics of Fluoroquinolones

The original work on the pharmacodynamic properties of the fluoroquinolone antibiotics was done without consideration of the protein binding and the free fraction of the antibiotic in question. The degree of protein binding with the older quinolones, ciprofloxacin, norfloxacin, ofloxacin were similar, ranging from 20 – 40%, (48) (42) causing little effect on the comparison of pharmacodynamic parameters within the group. Some, but not all, of the newer fluoroquinolones showed higher protein binding as evidenced by the protein binding of trovafloxacin (70%) (42), garenoxacin (87%) (27) and gatifloxacin (20%) (27). Studies by Synphar have shown that SYN 1193 is 90 -92 % protein-bound in humans and 80 – 82% protein-bound in rabbits (49).

Only the free fraction of the antibiotic diffuses across membranes to the site of infection and binds to the site of action in the bacterial cell (32). The fluoroquinolones bind primarily to albumin. In 2000, Craig presented an abstract showing the effect of degree of protein binding on the total AUC_{24}/MIC ratio and free AUC_{24}/MIC ratios of the quinolones including the new 6-desfluoroquinolone, garenoxacin, against *S. pneumoniae*. Taking protein binding into account, the free AUC_{24}/MIC ratio ($fAUC_{24}$/MIC) required for efficacy against *S. pneumoniae* is approximately 30 - 35 for all quinolones studied (see **Figure 1.1**). Unfortunately, this was not published and did not appear in the literature until the abstract was cited by Ambrose in 2003 (32). Since the year 2000, many authors have reported either an unspecified AUC_{24}/MIC or free concentrations ($fAUC_{24}$/MIC) in the literature regarding the pharmacodynamics of the fluoroquinolones. It is quite surprising how many articles are still published without a designation of free or total concentrations. It has now become generally accepted that a *f*AUC/MIC ratio of > 30 is required for the treatment of *S. pneumoniae*. On the basis of this work, a minimum *fAUC/MIC* of >30 had been suggested for the treatment of gram-positive organisms with fluoroquinolones.

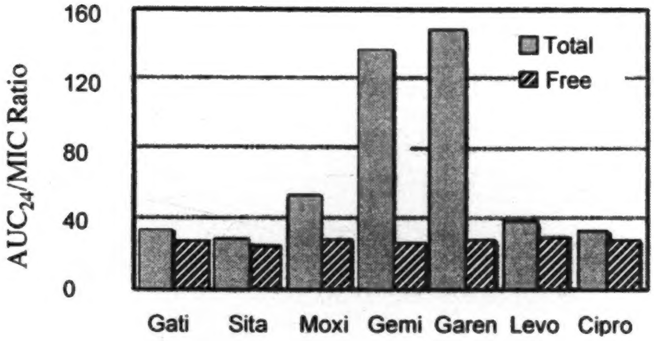


Figure 1.1. The AUC_{24}/MIC of total and free drug concentrations associated with effective treatment in animal models of infection with *S. pneumoniae* treated with 7 quinolone antibiotics. Taking the free fraction into consideration the $fAUC_{24}$/MIC ratio correlated with successful treatment is between 30 and 35 for all quinolones studied. Gati – gatifloxacin, Sita = sitafloxacin, Moxi = moxifloxacin, Gemi = gemifloxacin, Garen = garenoxacin, Levo = levofloxacin, Cipro = ciprofloxacin (50) [Ambrose, 2003 #746]

Fluoroquinolone Activity Against Staphylococcus aureus

In 2002, Andes and Craig studied the pharmacodynamic effects of gatifloxacin in the murine thigh model with *S. aureus, S. pneumoniae and Enterobacteraciae*. In this study, an AUC_{24}/MIC of 50 ± 20 was the PK/PD parameter that best correlated with efficacy with *S. aureus*. Methicillin, penicillin or ciprofloxacin resistance did not alter the magnitude of the AUC_{24}/MIC required for efficacy (51). In 2003, Ambrose et al. reported that the magnitude of the *f*AUC_{24}/MIC required for efficacy of fluoroquinolones was higher with *S. aureus* than *S. pneumoniae*. Studied in the murine thigh infection model, *f*AUC_{24}/MIC ratios greater than 60 – 80 were associated with a 90% reduction in bacterial density or greater than 90% animal survival (32). In the same year, Craig and Andes also reported that the AUC_{24}/MIC ratios required for efficacy in the murine neutropenic thigh model infected with 6 strains of *S. aureus* were 81 ± 37 with garenoxacin, a new des-F(6)-quinolone (52). Methicillin, penicillin or ciprofloxacin resistance did not alter the magnitude of the AUC_{24}/MIC ratio required for efficacy in this study. With these studies, it became apparent that the pharmacodynamic indices for fluoroquinolones correlated with the effective treatment of gram-positive infections vary with the type of organism. While *f*AUC_{24}/MIC of > 30 of a fluoroquinolone may be appropriate for the treatment of a pneumococcal infection, a higher *f*AUC_{24}/MIC ratio of at least 50-80 would be required for the treatment of a staphylococcal infection.

Pharmacodynamic Parameters of Fluoroquinolones Required for Resistance Counterselection

The fluoroquinolone antibiotics have been shown to target two primary sites in the bacterial cell, DNA gyrase encoded by *gyrA* and *gyr B* and topoisomerase IV encoded by *parC* and *parE* (*grlA* and *grl B* in staphylococci). In gram-negative bacteria DNA gyrase is the favoured target while in gram-positive bacteria topoisomerase IV is the favoured target. Some fluoroquinolones such as clinafloxacin appear to target both DNA gyrase and topoisomerse IV equally(53).

Resistance in staphylococci usually develops through mutations in the quinolone resistance determining region (QRDR) of gyrA, and / or grlA. Within a given gene many different mutant alleles can occur leading to resistance (54). In addition, in gram-positive and gram-negative organisms, over-expression of efflux pumps may lead to resistance. In gram-positive bacteria, multi-drug efflux pumps such as NorA in staphylococci and PmrA in pneumococci lead to quinolone resistance. Low-level resistance in gram-negative organisms has also been shown to occur through the development of altered membrane porins.

In 1998, Thomas et al. studied the development of resistance in a group of 107 critically ill patients with lower respiratory tract infections associated with 128 pathogens. The emergence of resistance occurred in 25% of the patients. An AUC_{24}/MIC of <100 was associated with a higher incidence of the development of resistance. Emergence of resistance occurred in 82% of cases with an AUC_{24}/MIC ratio of <100 and only 9% of cases when the ratio was ≥100 (55). Although this study has been criticized due to the number of various pathogens and antibiotics involved, it has remained a landmark study. A re-evaluation of this data showed that a peak/MIC ratio of ≥ 8 was just as protective of the development of resistance as an $AUC_{24}/MIC \geq 100$ (36, 56).

In a more recent study in 2003, Jumbe et al., studied the development of resistance using an in vitro model of *P. aeruginosa* treated with levofloxacin. The investigators found that the total AUC_{24}/MIC of ≥157 (equivalent to a free AUC_{24}/MIC ratio of 110) was associated with the prevention of amplification of existing mutant subpopulations (57), similar to the earlier findings by Thomas.

Tam et al. studied the development of resistance with *K. pneumoniae* treated with garenoxacin (58). In vitro experiments determined that a $f\text{AUC}_{24}$/MIC ratio of 88.3 was necessary for total bacterial killing and the prevention of regrowth of resistant subpopulations. Methicillin-susceptible *S. aureus* treated with garenoxacin, required an $f\text{AUC}_{24}$/MIC of ≥ 67 to sustain killing of the entire population and prevent selection of resistant subpopulations. For ciprofloxacin-susceptible MRSA, garenoxacin $f\text{AUC}_{24}$/MIC ratios of ≥144 were required to suppress selection of resistant subpopulations and for ciprofloxacin-resistant MRSA, garenoxacin $f\text{AUC}_{24}$/MIC ratios of ≥ 447 were required to suppress the emergence of resistant subpopulations (59). These varying requirements of AUC_{24}/MIC ratio for prevention of selection of resistance in strains of *S. aureus* with differing level of resistance from MSSA to ciprofloxacin-resistant MRSA are most interesting in view of the findings by Craig et al. that methicillin or ciprofloxacin resistance did not affect the AUC_{24}/MIC ratio required for efficacy.

In 2004, Firsov et al. (60) used an in vitro model to determine the pharmacodynamic parameters of ABT 492 and levofloxacin required to prevent the selection of resistant *S. aureus*. Three clinical isolates were used, two ciprofloxacin-sensitive and one ciprofloxacin-resistant. AUC_{24}/MICs ratios of 240 and 200 were found to be protective against the selection of resistant *S. aureus* with ABT 492 and levofloxacin respectively. No difference was mentioned regarding varying AUC_{24}/MIC ratios with ciprofloxacin-sensitive and ciprofloxacin-resistant strains.

Fluoroquinolone Mutant Prevention Concentration

In the past few years, a number of publications have outlined the concept of mutant prevention concentrations (MPC), a novel, in vitro measurement of fluoroquinolone potency. The mutant prevention concentration is the concentration of antibiotic that prevents the growth of next-step mutants of a bacterial strain. It is determined by plating 10^{10} bacterial cells onto plates with varying concentrations of the quinolone and determining the concentration at which no growth occurs (61, 62). For the quinolone antibiotics, it is, in essence, the antimicrobial drug concentration threshold that would require an organism to simultaneously possess two resistance mutations in order for the organism to grow in the presence of the drug (61).

When *S. aureus* was plated on agar containing increasing concentrations of fluoroquinolone, colony numbers exhibited a sharp drop, followed by a plateau and a second sharp drop. Mutants were not recovered above those required for the second drop, thereby defining a mutant prevention concentration **Figure 1.2** (63) The C8- methoxy fluoroquinolones were more effective than C8-hydrogen fluoroquinolones in lowering the MPC with mycobacteria and *S. aureus* (64) .

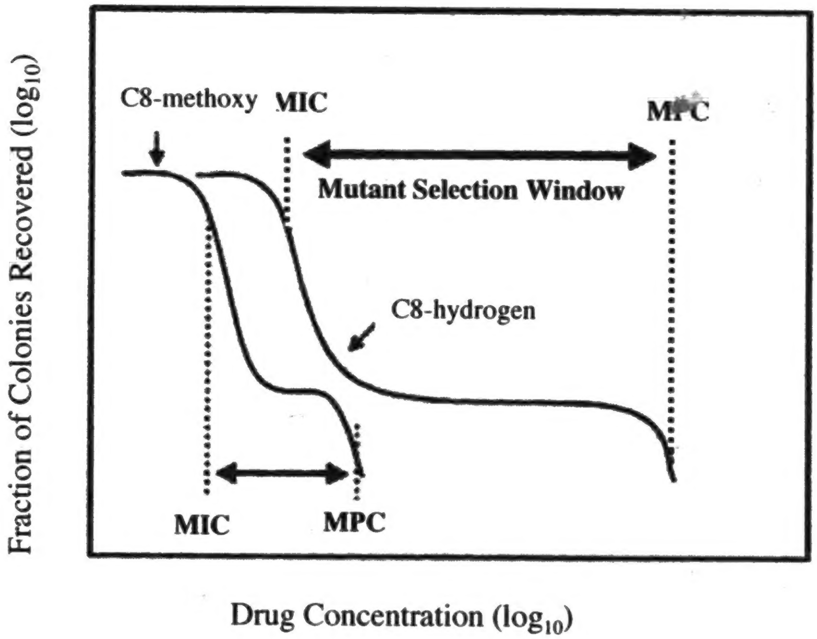

Fraction of Colonies Recovered (log10)
C8-methoxy
MIC
MPC
Mutant Selection Window
C8-hydrogen
MIC
MPC
Drug Concentration (log10)

Figure 1.2 **Mutant Selection Concentration (64)**

Many in vitro studies have been done to determine the effect of varying concentrations of the fluoroquinolone antibiotics and the selection of mutant strains. Out of this has arisen the concept of a mutant selection window (MSW) – the concentration of drug that is between the concentration that begins to inhibit growth of the organism (approximately the MIC), up to the mutant prevention concentration (61). With antibiotic concentrations below the MIC, the selective amplification of resistant mutants is not likely to occur as both susceptible and resistant organisms grow and compete within the environment, however, treatment failure with concentrations below the MIC is very likely to occur. With antibiotic concentrations above the MPC, wild-type bacteria and first-step mutants are killed resulting in successful treatment and prevention of the development of resistance. However, with antibiotic concentrations within the mutation selection window (MSW) (the concentration between the minimum inhibitory concentration and the mutant prevention concentration), growth of wild-type organisms are inhibited allowing natural selection and enrichment of first-step mutants that have developed one mutation by chance or organisms with a pre-existing mutation. Such mutations occur at a rate of 10^{-6}-10^{-8} and therefore are likely to occur in the clinical setting of infection. If these organisms with first-step mutations are not destroyed, they may go on to develop a second-step mutation and much higher MICs for the drug in question. A graph showing the MSW in relation to the concentration vs. time curve is depicted in **Figure 1.3.** It is estimated that each mutation increases the MIC by 4-8 fold and resistance develops in a step-wise fashion (53).

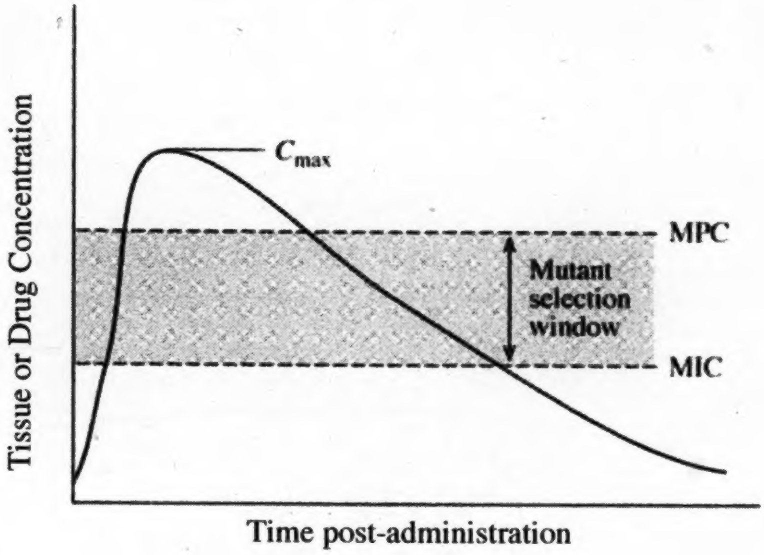


Figure 1.3. **Mutant Selection Window (MSW)** From Drlica et al. 2003(61).

The MICs and MPCs of various strains of MRSA have been determined by Allen et al., MPC concentrations were found to be approximately 2 – 4 fold higher than the MIC (65).

Although the concepts of mutant selection window and MPC have been demonstrated in vitro experiments, it is not known how they apply to the clinical setting. For example, must plasma concentrations remain above the MPC throughout the entire dosing interval, or 50% of the dosing interval to be effective or will an AUC/MPC or peak/MPC ratio or Cssavg>MPC correlate with best with clinical efficacy? Recently, in an in vitro experiment by Campion studying the emergence of resistance, *S. aureus* was exposed to varying simulated pharmacokinetic profiles of levofloxacin. Regimens producing average steady-state concentrations (Cavg ss) of levofloxacin above the MPC appeared to eradicate low-level resistant variants and prevent the emergence of resistance (66). In a similar study with ciprofloxacin-sensitive *S. aureus*, ciprofloxacin regimens designed to maintain ciprofloxacin concentrations above the MPC appeared to eradicate low level-resistant variants and prevent the emergence of higher levels of resistance (67).

Firsov et al. studied the concept of MSW using four fluoroquinolones (ciprofloxacin, levofloxacin, gatifloxacin and moxifloxacin) against *S. aureus* using an in vitro dynamic model. It was found that with all four fluoroquinolones, there was no change in the MIC of *S. aureus* at the highest AUC_{24}/MIC ratios ranging from 201- 244 where drug concentrations exceeded the MPC for most of the dosing interval. Moxifloxacin was found to be most effective in protecting against mutant selection at clinical doses. It was calculated that the required AUC_{24}/MIC ratios of 201-244 could be achieved with only 66% of the usual 400 mg dose of moxifloxacin, however, the usual 500mg doses of levofloxacin and 400mg doses of gatifloxacin would need to be doubled and ciprofloxacin doses of 500 mg b.i.d. would need to be quadrupled (68).

In an vitro study in which *S. aureus* was exposed to gatifloxacin concentrations to simulate normal (NEK) and impaired excretion kinetics (IEK), Firsov found that with both simulations, significant increases in MIC were observed at those AUC_{24}/MICs that correspond to gatifloxacin concentrations within the MSW over most of the dosing interval (>25%). No increases in MIC were observed at the smallest AUC_{24}/MIC when the simulated C(max)s were close to the MIC resulting in minimal bacterial killing. At the highest AUC_{24}/MICs (310 and 160 h, respectively) gatifloxacin concentrations exceeded the MPC for most of the dosing interval and maximal antimicrobial effect with no increases in MIC occurred (68). This study supports the concept of a MSW and the concept that mutant selection will not occur with concentrations below the MIC or above the MPC, but will occur within the MSW.

Further animal and human studies are required to prove the concept and determine the appropriate clinical application of MPC and MSW in the in vivo setting. With continuing problems with the development of resistance and continued research, it may well be proven with time, that the most important pharmacodynamic parameter that we must strive to meet in designing therapeutic antibiotic regimens will be the time above the MPC (t>MPC), AUC_{24}/MPC ratio, C_{max}/MPC, C_{ssav}/MPC or AUC_{24}/MSW that correlates best with minimal mutant selection and enhancement and prevention of the development of resistance.

Vancomycin

Vancomycin, a glycopeptide antibiotic, is considered to be the "gold" standard in the treatment of gram-positive infections, particularly MRSA. In these studies vancomycin will be used as the gold standard positive control used for comparison of efficacy of SYN 1193 in the rabbit model of peritonitis in peritoneal dialysis.

Pharmacodynamics of Vancomycin

In comparison to the fluoroquinolone antibiotics, little work has been done to evaluate the pharmacodynamic parameters that correlate with therapeutic efficacy and suppression of emergence of resistant subpopulations with vancomycin or the glycopeptide antibiotics. Studies done have reported conflicting results and currently which pharmacodynamic parameters of vancomycin best correlate with efficacy are controversial (30).

Although vancomycin appeared on the market in the 1950's, it was not until the early 1981, with the emergence of methicillin-resistant *staphylococci* and increasing vancomycin use, that the first monograph guiding vancomycin dosing was published by Moellering et al. (69), followed by two others in 1984 by Matzke (70).

and 1988 by Lake (71) along with an in depth study of the renal handling of vancomycin by Golper et al. in 1988 (72).

The guidelines published by Moellering et al. were based on the concentrations expected with a 2 g/day dose in a 70 kg man rather than concentrations correlated with efficacy or toxicity (73). These guidelines soon, as Moellering put it, "took on a life of their own" (73) and routine monitoring of vancomycin concentrations became the standard practice.

In the mid 1990's routine monitoring of vancomycin concentrations came into question (73-77). Most authors agreed that routine monitoring of vancomycin was not necessary in most patients, that there was little evidence to support a correlation between serum concentrations and efficacy. Nephrotoxicity and ototoxicity of vancomycin when used alone was questioned. Monitoring was recommended in a few specific groups of patients at higher risk for the development of toxic or sub-therapeutic concentrations. In such cases, the measurement of trough concentrations, only, was recommended with the suggested (78) therapeutic range from 5 – 15 μg/mL. In 1995, Mulhern et al. found that trough serum vancomycin concentrations <12 μg/mL were associated with relapse of Gram-positive peritonitis in peritoneal dialysis patients (79). In a study of 273 patients with Gram-positive bacteremia in 1995, Zimmerman et al. found that patients were more likely to become afebrile if vancomycin peak and trough concentrations were 20 μg/mL and 10μgmL or greater respectively. Mean trough concentrations of >20 μg/mL (mean 23.2 μg/mL) were more likely to be associated with nephrotoxicity. (80).

Emerging resistance, increasing use of vancomycin and the development of teichoplanin lead to renewed interest in the pharmacodynamics of the glycopeptides in the late 80s and early 90s, but studies are still few.

Early studies of the pharmacodynamics of vancomycin in 1987 reported that the AUC_{24}/MIC ratio and AUC plus peak were the pharmacodynamic parameters that correlated best with efficacy. Unfortunately, these findings were only published in abstract form as reported by Craig (30).

Peetermans et al., in 1990, compared in vitro and in vivo rabbit models of endocarditis to show that bacterial killing with vancomycin and teichoplanin was concentration independent once a threshold of one to two times the MIC was reached. The in vivo response correlated well with the in vitro model. In the same year, Chambers et al. investigated the efficacy of teichoplanin given I.M. or I.V. in a rabbit model of endocarditis infected with *S. aureus* and *Streptococcus sanguis*. This group found I.M administration to be more efficacious than I.V. administration concluding that high peaks were unlikely to be an important determinant of efficacy and that the more relevant concentration in serum may be the trough.

Duffull et al. in 1994 used a dynamic in vitro model to simulate peak concentrations of 48 μg/mL as a single dose, and 30 μg/mL dosed q12h as well as constant concentrations of 16 and 8 μg/mL. Rate and extent of bacterial killing were identical with all four dosing regimens suggesting the absence of a concentration-dependent bactericidal effect. The authors suggested that it may be more rational to aim for constant concentrations above the MBC than the classic peak of 20-40 μg/mL and trough of 5 – 10 μg/mL. Doing so would likely reduce toxicity and save 50% of drug costs (81).

In 1996, Larsson et al. studied the effect of varying peaks of vancomycin of 5, 10, 20 and 40 μg/mL against *S. aureus* using an in vitro system with a simulated $t_{1/2}$ of 6 hours under aerobic and anaerobic conditions. This group reported that varying the concentration of vancomycin had no effect on the rate or extent of bacterial killing, however, killing was much more effective in an aerobic environment.

In 1997, Knudsen et al. found t >MIC and C_{max} correlated best with efficacy of the glycopeptides vancomycin and teichoplanin in the mouse peritonitis model infected with penicillin-resistant *S. pneumoniae*. When each drug was considered separately, t > MIC was the most constant parameter. In further studies, in 2000, this group found that a mean $C_{max\text{-}free}$/MIC of 5-6 and AUC_{24}/MIC ratio of vancomycin correlated best with efficacy in an immunocompetent mouse peritonitis model infected with *S. aureus* or *S. pneumoniae*. However, all three parameters, t >MIC_{free}, $C_{max\text{-}free}$/MIC and AUC_{24}/MIC, were closely correlated with efficacy in this study. Giving vancomycin in one full dose rather than 2 half-doses improved survival in this study.

In a review of pharmacodynamics and antimicrobial agents in 1998, Craig reported (29) that vancomycin displayed minimal concentration-dependent killing with saturation of bacterial killing at 4 -5 x MIC. The extent of killing was dependent on time of exposure - a common characteristic of ß-lactams, vancomycin, clindamycin and macrolides. It was also reported that the AUC_{24}/MIC ratio was the main PK/PD parameter correlating with the therapeutic efficacy possibly due to longer PAE. In a more recent review by Craig, it was reported that glycopeptides exhibit time-dependent killing but produce prolonged persistent effects. Dosing frequency is not the major factor in the efficacy of these drugs. AUC_{24}/MIC ratio is primary parameter to correlate with in vivo efficacy (56).

In 2000, Lacy et al. compared the duration of serum bactericidal activity (SBA) of vancomycin against MRSA and methicillin-resistant coagulase negative *S. aureus* with MICs of 2 or 4. Vancomycin 1 g q12h or q24h h was administered to healthy volunteers. Serum concentrations were measured at steady state. SBA, defined as serum bacterial titres of 1:2, were achieved for 10 -12 hr with the q12h regimens and 10-16 hr for MRSA and 8-10 hr for MR-CNS during the q24h regimens. It was concluded that for those with good renal function, q12h dosing would be most appropriate for isolates with MICs of 4.

In 2003, Pavie et al. studied vancomycin and teicoplanin in the treatment of a susceptible strain of *S. aureus* and a strain with reduced susceptibility in the rabbit model of endocarditis. Vancomycin dosing regimens of 400 mg q12h and 500 mg q12h resulted in peaks of 39 ± 12 and 45 ± 8 μg/mL and troughs of 12 ± 3 and 20 ± 5 μg/mL. Both dosing regimens were equally effective against either strain of *S. aureus*. The AUC_{24}/MIC ratios ranged from 84 to 372. It was concluded that an AUC_{24}/MIC ratio of approximately 80 was associated with effective treatment. It must be considered that this study was not designed using dose fractionation to determine which pharmacodynamic parameter is most closely correlated with effective treatment. In this study, peak/MIC ratios and troughs are high with both dosing regimens and either may be a determinant of effective treatment. Although it was concluded that an AUC_{24}/MIC ratio of 80 was associated with effective treatment, no regimen achieving an AUC_{24}/MIC ratio less than 80 was studied and no treatment failures occurred to delineate a minimum AUC_{24}/MIC. A smaller AUC_{24}/MIC ratio may have been effective or, the AUC_{24}/MIC ratio may not be the determining factor (82).

In two clinical studies, in 2000 and 2004, to elucidate the most important pharmacodynamic determinant of vancomycin response in ventilator-dependent pneumonia and *S. aureus* lower respiratory tract infection, Moise-Broder et al. found that clinical and bacteriological response to vancomycin therapy was superior in patients with AUC_{24}/MIC ratios of ≥ 350 and 400 (83, 84). No relationship was found between vancomycin %t >MIC and infection response as %t >MIC was identical at 100% in patients cured and treatment failures. Bacterial eradication was more rapid with an AUC_{24}/MIC of ≥ 400. Craig has pointed out that this AUC_{24}/MIC ratio may be high due to the effect of protein binding which is approximately 50-65% humans, however, it is more likely due to the relatively poor penetration of vancomycin into respiratory secretions such as epithelial lining fluid (ELF). Concentrations of vancomycin in ELF are normally only 10 -18% increasing to 25% in inflammation (30).

Effect of Protein Binding on Vancomycin Pharmacodynamics

Most studies of vancomycin pharmacodynamics have been reported without regard to protein binding. Tests used routinely in laboratories for therapeutic drug monitoring measure total vancomycin concentrations. In 1997, Knudsen et al. found the protein binding of vancomycin and teicoplanin in the mouse to be 20 – 28% and 90 – 94% respectively. In the human, protein binding was 25 – 41% for vancomycin and 89 – 95% for teicoplanin (85). It is interesting to note, that although all possible parameters such as $C_{max\ free}$/MIC and T>MIC free were calculated in this study, t>MIC and C_{max} (total concentration) were the parameters that best correlated with efficacy in the mouse peritonitis model (85). However, the Knudsen paper in 2000 reported that the pharmacodynamic parameters associated with efficacy were T/ MIC(free) and Cmax free/MIC. In 2000 and 2004, Moise-Broder reported the pharmacodynamic parameters AUC_{24}/MIC ratios of 350 and 400 correlated with efficacy (total concentration), however in the discussion in the 2004 paper it was noted that if protein binding estimated to be 60% was taken into consideration the AUC_{24}/MIC free may be closer to the value of 125 associated with efficacy of other antibiotics.

It is clear that there is no consensus as to which pharmacodynamic parameters correlate best with efficacy of vancomycin. Many parameters, Cmax, Cmax/MIC, Cmax/MIC free, AUC_{24}/MIC, %T>MIC have been correlated with efficacy in various studies. Results vary from parameters of %T>MIC which would give the impression that vancomycin displays concentration independent pharmacodynamics to Cmax/MIC which would give the impression that vancomycin displays concentration dependent pharmacodynamics. Studies vary in design from in vitro models, animal models to human studies. Variables such as protein binding and immune response and site of infection may account for some of the discrepancies in these studies. Good dose fractionation studies with varying simulated half-lives have not been done to clearly define the pharmacodynamics of vancomycin. **Table 1.4** outlines a number of the studies done to determine the pharmacodynamics of vancomycin.

Table 1.4, Studies done to elucidate the pharmacodynamics of vancomycin.

Author	Model	Peak/MIC	AUC_{24}/ MIC	T>MIC	Peak/MIC free	Dosing
Ebert 1987			AUC_{24}/ MIC + peak			
Peetermans 1990	In vitro + Rabbit endocarditis	Conc. Independent -2 X MIC max				
Chambers 1990	Rabbit Endocarditis					I.M. > I.V.
Duffull 1994	In vitro	No peak Effect		Suggests T>MBC		
Larssen 1996	In vitro	No peak effect				
Knudsen 1997	Mouse peritonitis	C_{max}		T>MIC		
Knudsen 2000	Mouse peritonitis				Cmax/MIC Free > 5-6	o.d. >b.i.d.
Dudley 1999			AUC_{24}/ MIC 86-460 50% max killing			
Lacy 2000	Healthy Human					Q12h >q24h
Pavie 2003	Rabbit endocarditis		AUC_{24}/ MIC >80			
Moise-Broder 2000	Human Ventilator pneumonia		AUC_{24}/ MIC >350			
Moise-Broder 2004	Human *S. aureus* pneumonia		AUC_{24}/ MIC >400			

Biofilm

Biofilm frequently complicates the treatment of peritonitis and catheter-related infections resulting in treatment failure and the need to remove or replace the PD catheter.

Biofilms have been shown to play a significant role in the development of many infections such as pneumonia caused by *Pseudomonas aeruginosa* in patients with cystic fibrosis, endocarditis, prostatitis, periodontal disease and osteomyelitis as well as infections involving foreign substances such as intravascular catheters, peritoneal catheters, urinary catheters, pacemakers, intrauterine devices and artificial joints. In human medicine, it has been estimated that 65% of nosocomial infections are biofilm associated costing the health care system billions of dollars(86). Central venous catheters removed from patients have been shown to be universally colonized by biofilms (87). Biofilms may develop rapidly. In vitro studies by Dasgupta et al. have shown that biofilm can develop on a silicone catheter within 4 to 24 hrs of inoculation in dialysate [Dasgupta 1994 #192].

Biofilm develops as a survival technique of bacteria or fungi in response to stress. Bacteria adhere to and grow on foreign substances including catheters, pacemakers, artificial or damaged heart valves and artificial joints (86). Specific gene products are required for the initial association of bacteria with a surface. Dozens of new genes are turned on and others are turned off as bacteria move onto a surface (88).

Bacterial motility seems to be critical in the early stage of biofilm formation – bacteria use flagellar, twitching and gliding motility mechanisms to grow together in nascent cluster (89). Further organization of the biofilm into complex structures is regulated by the exchange of chemical signals between cells in a process known as quorum sensing. (89) For example, the expression of the *agr* quorum-sensing system in *S. aureus* is known to affect virulence factor production and may also affect antibiotic resistance and energy metabolism through complex interactions with other staphylococcal gene regulators (90).

Once adherent to a foreign surface, a phenotypic change takes place in the bacterial cell with altered gene expression (87). For example, the gene (algC) involved in alginate (exopolysaccharide) synthesis of *Pseudomonas aeruginosa* is up-regulated within minutes of adhesion to a solid surface (87). Altered gene expression may also result in the development of antimicrobial resistance.

Once adherent to a foreign surface, the bacterial cell begins to secrete a sticky substance composed of extracellular polymers that protect the bacteria cell from the hostile environment. The bacteria begin to grow and divide slowly in this environment forming microcolonies encased in a protective matrix. The exact composition of the matrix varies with the type of organism but the extracellular polymeric substance (EPSs) consists primarily of polysaccharides and serves to protect the bacterial growth (87). This matrix has been shown to be quite complex in nature, consisting of many channels through a matrix allowing diffusion of nutrients in, and waste products out of the biofilm. The matrix is highly hydrated, composed of 95% water, and is tenaciously bound to the underlying surface. Adherent bacteria within this matrix are termed "sessile" while free-floating bacteria are termed "planktonic". Sessile bacterial cells within matrix-enclosed fragments may break off from a biofilm and circulate in body fluids with all the resistance characteristics of the parent community (87) or they may be dispersed from the biofilm, in their planktonic form (**Figure 1.5**). Dispersed bacteria usually rapidly become susceptible to antibiotics again (89).

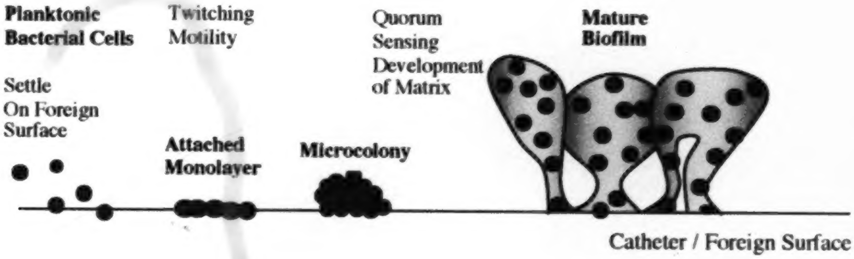

Planktonic Bacterial Cells
Twitching Motility
Quorum Sensing Development of Matrix
Mature Biofilm
Settle On Foreign Surface
Attached Monolayer
Microcolony
Catheter / Foreign Surface

Figure 1.4 Process of Biofilm Formation. Adapted from Costerton et al 1999. (91)

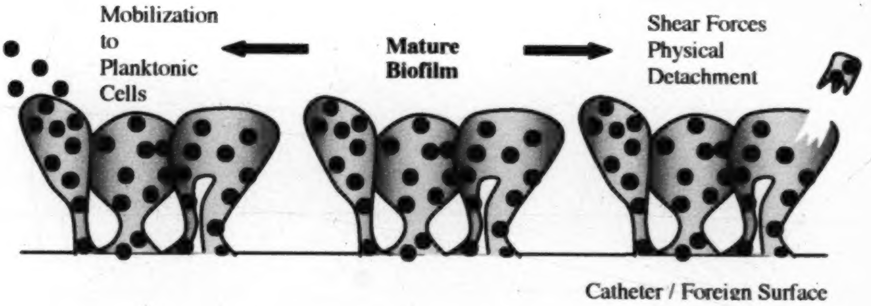

Mobilization to Planktonic Cells
Mature Biofilm
Shear Forces Physical Detachment
Catheter / Foreign Surface

Figure 1.5 Detachment and Dispersal of Planktonic and Sessile Forms of Bacterial Cells from Biofilm. Adapted from Costerton et al. 1999 (91).

Biofilm has recently been defined as a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced (87). Such organisms exhibit an altered phenotype with respect to growth rate and gene transcription (87).

Bacteria within an organized biofilm are resistant to biocides and antimicrobial agents. Bacteria protected in this environment may survive treatment and serve as a nidus for recurrent or chronic infections. Resistance may be due to one or more of the following: delayed penetration of the antimicrobial agent through the biofilm matrix, altered growth rate of the bacterial cell in the biofilm, altered gene expression, increased plasmid transmission or protection from the host immune system (89) (92, 93).

The effect of the biofilm matrix on diffusion of antimicrobial agents varies. Tetracycline has been shown to completely diffuse through a biofilm matrix in 7.5 – 10 minutes following exposure (94). Ampicillin has been shown to diffuse freely through biofilm formed by *Klebsiella pneumoniae* (95), while the diffusion of ciprofloxacin was reduced but not completely blocked (95). However, ampicillin was destroyed in the layers of biofilm with strains of *Klebsiella pneumoniae* that produced ß-lactamase. Diffusion of the cationic aminoglycosides through biofilm is impeded as the positively charged antibiotics bind to the negatively charged polymers in the biofilm matrix (96).

The microenvironment varies throughout the biofilm resulting in microscale gradients in nutrient concentrations (89). Organisms may grow and divide more slowly in biofilm due to nutrient depletion. Antibiotics such as the ß-lactams, which kill only growing bacteria, may not be effective in this environment (89). Waste products may accumulate in the layers of biofilm and oxygen may be depleted leading to an anaerobic environment that may decrease the activity of the aminoglycoside antibiotics.

Gene expression of sessile bacteria in biofilm is altered and may lead to production of the biofilm matrix or antibiotic resistance. Whitely et al. compared gene expression of *P. aeruginosa* in planktonic bacteria and mature biofilm. He found that 73 genes showed differential expression in mature biofilm - 34 genes were activated and 39 repressed in the biofilm population – some of the genes activated or repressed in biofilm were shown to affect biofilm development and antibiotic sensitivity in *P. aeruginosa*. Further studies by Whiteley et al. found that 20 genes were differentially expressed in *P. aeruginosa* in biofilm resulting in MICs that were seven times the minimum inhibitory concentration of planktonic cells (88)

Killing of bacteria within a mature biofilm may require antimicrobial doses 10 – 1000 times higher than those required to kill the planktonic form of the same organism (92, 97, 98). In an in vitro study by Anwar et al., using a modified Robbins Device (MRD), planktonic cells of *P. aeruginosa* were killed by exposure to tobramycin 10 μg/mL in 2 hr. Bacterial cells of the same strain in young biofilm 2 days after colonization were more resistant with approximately 40% of the adherent cells viable after exposure to tobramycin 10μg/mL for 5 hr. Only 1.5% remained viable after increasing the concentration of tobramycin to 20 μg/mL for 5 hr. Older biofilm, 7 days after colonization, were much more resistant with 15% remaining viable after exposure to tobramycin 200 μg/mL for 5 hours. When the biofilm was scraped from the adherent surface, the planktonic cells were again sensitive to tobramycin 5μg/mL (97).

In biofilm, bacteria are held in close proximity. This environment is ideal for the transfer of plasmids from one organism to the next resulting in the spread of multi-antibiotic resistance. Rates of plasmid transfer have been shown to be several fold higher in biofilms than in liquid cultures of the same organisms (89).

Bacteria within a biofilm are protected from the circulating immune system by the EPS. Antibodies present in the blood stream are unable to diffuse through the biofilm matrix. Antibodies, complement and invading neutrophils may result in collateral damage of adjacent tissues in their misguided attack (92). Bacteria in their sessile form have also been shown to be resistant to the oxidative burst following phagocytosis by neutrophils.

Routine MIC testing that measures the minimum inhibitory concentration of an antimicrobial agent against a bacterial cell in its planktonic state is of limited value in determining the effect of the antimicrobial agent on biofilm (99). In response to this concern, Ceri et al., developed the Calgary Biofilm Device (CBD). Using this device, biofilms are grown on small "pegs" which fit into a standard 96-well plate apparatus used to test for antimicrobial or biocide sensitivity of bacteria in their sessile form. This device determines he minimum biofilm eradication concentration (MBEC), which is the concentration of antimicrobial agent required to kill a bacterial biofilm (100, 101). Using this device, Olson et al. tested varying strains of gram-positive and Gram-negative organisms associated with pathogenic bacteria associated with veterinary infections. For many strains, great discrepancies were found between the MIC of the planktonic form and MBEC of the sessile form of the same bacterial strain. For example, the MIC of penicillin G against *S. aureus* was found to be 2 μg/mL in its planktonic form while the MBEC of its sessile form was > 1024 μg/mL. This strain of *S. aureus,* while sensitive to several antibiotics tested against its planktonic form, was resistant to all antibiotics tested in its sessile form (penicillin G, cloxacillin, streptomycin, ceftiofur, tetracycline, ampicillin, and oxytetracycline). The MIC of gentamicin against *E. coli* in its planktonic form was 32 μg/mL, while in its sessile form, the MBEC was 512 μg/mL. When the same strain of E. coli was tested with enrofloxacin, the MIC and the MBEC were the same <2 μg/mL. Most antibiotics tested were effective in inhibiting planktonic bacterial growth at low concentrations and the bacteria would be considered sensitive based upon NCCLS breakpoints. Only a limited number of antibiotics were effective in killing biofilm bacteria at relatively low concentrations.

Studies of PDE in patients with peritonitis were done to determine the MIC and MBEC of infecting organisms. The standard MIC assay following NCCLS guidelines revealed that 62.5% of coagulase-negative *S. aureus* (CNS) were resistant to first generation cephalosporins. Resistance to vancomycin was not seen with MIC testing. On MBEC testing, 75% of CNS were resistant to first generation cephalosporins and 17% resistant to vancomycin. No resistance was noted with vancomycin and rifampin 1:1 combination using MIC or MBEC determinations (102). The group made similar conclusions in comparing the MIC and MBEC values of *E. coli* and *P. aeruginosa* isolated from PDE of patients with peritonitis. Seven strains of P. aeruginosa were considered sensitive to gentamicin and 1 strain resistant with MIC testing, while with MBEC testing, only one strain was determined to be sensitive, 2 strains of intermediate sensitivity and 5 strains resistant. These findings are consistent with the findings of Anwar et al. testing tobramycin sensitivities against planktonic and sessile forms of *P. aeruginosa* as previously described (97).

Rabbit Model

In these studies we will use a rabbit model to simulate peritonitis due to methicillin-resistant *S. aureus* in peritoneal dialysis.

The model includes an indwelling Silastic catheter of the type used in humans embedded along a subcutaneous tunnel. In humans, catheter-related infection may develop either through touch contamination during manipulations during dialysis exchanges, or as an exit site infection that tracks along the subcutaneous tunnel to the peritoneal cavity. In the model used in these studies, infection with a human strain of MRSA, MU7056 isolated from a patient with peritonitis, takes place at the exit site and travels along the subcutaneous tunnel surrounding the catheter, through the Dacron cuff and into the peritoneal cavity. Peritoneal dialysis exchanges impair host defenses and contribute to the development of peritonitis. Peritoneal dialysis exchanges with Dianeal can be performed on a schedule identical to those used in patients. Antibiotics will be administered into the intraperitoneal cavity with dialysis exchanges following the usual patient schedule. Blood and peritoneal fluid samples will be collected for culture, blood and PDE concentrations of antibiotic can be measured and tissue and catheter samples can be assessed for bacterial growth and the presence of biofilm.

Goal

The overall goal of this project is to investigate alternative approaches to vancomycin therapy in the treatment of gram-positive peritonitis in CAPD patients. A validated rabbit model of peritonitis in peritoneal dialysis will be used to determine pharmacokinetics, pharmacodynamic indices and efficacy of the investigational fluoroquinolone, SYN 1193, compared to vancomycin, the positive control, and placebo, in the treatment of MRSA peritonitis. The rabbit model has been previously validated by Dr. Dasgupta and co-investigators as a model of bacterial peritonitis with biofilm spread along the catheter surface in peritoneal dialysis (103), however, it has not been used as a model for the treatment of peritonitis with a human strain of *S. aureus* with integration of pharmacokinetic/pharmacodynamic studies.

Hypothesis

SYN 1193, an investigative fluoroquinolone, is as effective as the positive control vancomycin in the treatment of MRSA induced peritonitis in the rabbit model of peritonitis in peritoneal dialysis.

Chapter 2

Development of Sparfloxacin Assay

Sparfloxacin, (5-amino-1-cyclopropyl-6, 8-difluoro-1, 4-dihodro-7-(*cis-3,5-dimethyl-piperazinyl)-4-oxoquinoline-3-carboxylic acid*, Rhone Poulenc Rorer; **Figure 2.1**) is a member of the fluoroquinolone class of antibiotics. Sparfloxacin is known particularly for its Gram-positive activity, however it does have a broad spectrum of activity including many Gram-negative organisms as well (104).

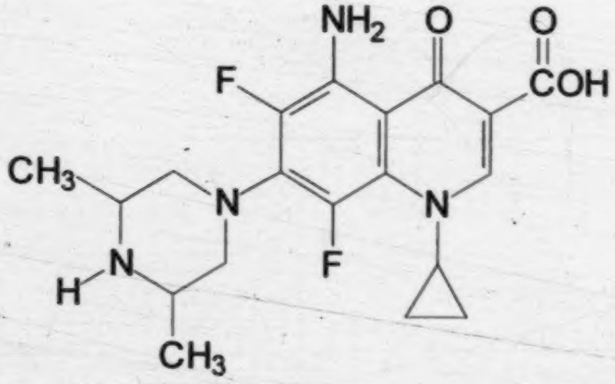


Figure 2.1. Chemical Structure Sparfloxacin

The determination of sparfloxacin concentration in biological fluids has previously proven to be difficult. A high performance liquid chromatographic (HPLC) assay for the determination of sparfloxacin concentrations has previously been reported. The method, however, requires direct injection of plasma (105). As a result, a rapid, convenient and sensitive assay that allows for extraction of sparfloxacin from biological fluids was developed.

Chemicals and Materials

Sparfloxacin was obtained from Rhone Poulenc Rorer, Ville St. Laurent, Quebec, Canada. The internal standard, ciprofloxacin was obtained from Bayer, Etobicoke, Ontario, Canada. Acetonitrile, analytical grade, was purchased from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada). Orthophosphoric acid was purchased from BDH (Edmonton, Canada), analytical grade triethylamine (TEA) was purchased from Mallinckrodt (Paris, KY, USA). HPLC grade ethyl acetate was obtained from BDH, Toronto Canada. HPLC grade water was glass double distilled in-house. Sorenson phosphate buffer solutions of varying pH were prepared as described in Geigy Scientific Tables (106).

Apparatus and Chromatographic Conditions

The HPLC system consisted of a Waters 6000A Solvent Delivery system (Waters, Mississauga, ON, Canada), Shimadzu SiL-9A Auto–injector (Shimadzu, Tokyo, Japan), a reversed-phase Partisil C8 5μ 250 mm x 4.6 mm analytical column (Phenomenex). Torrance, CA, USA), a Nova-Pak C8 Guard Column (Waters, MA, USA), a Shimadzu SPD-6AUV Spectrophotometric Detector (Shimadzu, Tokyo, Japan). A Model 3390 A Hewlett Packard integrator (Avondale PA, USA) was used to determine peak areas; the peak-area ratios of sparfloxacin/ciprofloxacin were used to quantify sparfloxacin in samples.

The mobile phase, acetonitrile 130 mL and 870 mL 2 mM orthophosphoric acid was mixed and degassed by filtration through a 0.45 μm membrane filter (Rose Scientific Products & Equipment, Edmonton, Alberta, Canada). Triethylamine 1.5 mL was added after filtration. The mobile phase was pumped at a flow rate of 1.0

mL/min at ambient temperature. The Shimadzu SPD-6AUV ultraviolet detector (Shimadzu, Tokyo, Japan) was set at 308 nm.

Preparation of Standard Solutions

Stock solutions of sparfloxacin 50 mg/L and ciprofloxacin 50 mg/L (as internal standard) were prepared with the addition of 5 mg of sparfloxacin or ciprofloxacin to 10 mL of 0.1 M NaOH. Sufficient pH 8 Sorenson phosphate buffer was added to produce a final volume of 100 mL and the solutions were stored a 4°C. These solutions were used to prepare standard curves.

Standard Calibration Curve Construction

The sparfloxacin calibration curve was constructed by spiking 0.5 mL aliquots of drug-free rabbit plasma or peritoneal fluid with sparfloxacin to yield concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 6.0, and 10.0 μg/mL.

As the internal standard, 100 μL of ciprofloxacin solution of 50 mg/mL was added to each tube.

The solutions were extracted twice with 5 mL ethyl acetate, vortex-mixed for 1 minute and centrifuged (Adams Dynac centrifuge, Clay-Adams, Parsipanny, NJ, USA) for 5 minutes at 1800 g. The organic layer was separated, transferred to clean tubes and evaporated to dryness (Savant Speed Vac concentrator-evaporator, Emerston Instruments, Scarborough, Canada) as described in the following section 'Sample Preparation'. The residue was reconstituted with 200 μL mobile phase. Varying aliquots were used for HPLC analysis depending on the concentration of sparfloxacin as listed in **Table 2.1**.

Table 2-1. **Sparfloxacin Standard Solutions for Construction of Calibration Curve**

Concentration µg/mL	Sparfloxacin	Volume and Dilution	Aliquot Volume (µL)
0.05	25 ng	50 µL of 1/100	120 µL
0.1	50 ng	100 µL of 1/100	120 µL
0.2	100 ng	200 µL of 1/100	120 µL
0.5	250 ng	50 µL of 1/10	120 µL
1.0	500 ng	100 µL of 1/10	100 µL
2.0	1 µg	200 µL of 1/10	75 µL
4.0	2 µg	40 µL of 1/1	75 µL
6.0	3 µg	60 µL of 1/10	50 µL
10.0	10 µg	100 µL of 1/10	50 µL
20.0	20 µg	200 µL of 1/10	25 µL

Sample Preparation

Ciprofloxacin 50 mg/mL as internal standard and 1.5 ml pH 8 Sorenson phosphate buffer were added to each 0.5 mL of biological fluid (plasma, peritoneal fluid, or urine), 100μL. The constituents were extracted with 5 mL of ethyl acetate, vortex-mixed for 1 minute, and centrifuged (Adams Dynac centrifuge, Clay-Adams, Parsipanny, NJ, USA) at 1800 g for 5 minutes. The organic phase was separated using a Pasteur pipette, transferred to fresh numbered tubes and evaporated to dryness without heat (approximately 30 minutes) (Savant Speed Vac Concentrator-evaporator, Emerston Instruments, Scarborough, Canada). The remaining aqueous phase was extracted a second time with 5 mL ethyl acetate, vortex-mixed for 1 minute, centrifuged for 5 minutes, the organic layer removed by Pasteur pipette and added to the residue in the evaporated tubes from the first extraction. The residue was reconstituted with 200 μL mobile phase and each HPLC sample tube was filled with 50 – 120 μL of reconstituted sample.

Extraction and Extraction Efficacy

To determine the optimal conditions for extraction of sparfloxacin, samples were extracted in triplicate at varying pH values (**Table 2.5**). The response to the latter extracted samples was compared to spiked samples of plasma that were directly injected without extraction.

A standard curve was prepared as described above. Thirty-six samples of 0.5 mL blank rabbit plasma were spiked with100 μL ciprofloxacin as internal standard and 50μL sparfloxacin stock solution 1/1 to obtain a concentration of 5 μg/mL. The pH was adjusted with 1.5 mL Sorenson buffer of varying pH 2-13 (106) to obtain triplicate samples with pH of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13. The pH of the samples was measured with a pH meter (Model 632 Brinkman, Metrohm Herisau, Switzerland). The samples were extracted twice with 5 mL ethyl acetate and the organic layer evaporated to dryness as described previously. The residue was reconstituted with 200 μL mobile phase. A 75 μL aliquot was injected. As comparators, 3 further samples were prepared with the addition of ciprofloxacin and sparfloxacin as above without pH adjustment or extraction before injection. Peak areas were calculated for sparfloxacin and ciprofloxacin. The peak area ratio of sparfloxacin to ciprofloxacin was determined for each sample and used to determine the concentration by comparison to the standard curve.

A plot of % extracted vs. pH was constructed to determine the optimal pH for extraction.

Assay Validation, Accuracy and Precision

To validate the assay, 3 standard curves and 3 sets of tubes of known concentrations of 0.04, 0.08, 5, and 10 μg/mL sparfloxacin in rabbit plasma with 100 μL ciprofloxacin 50 μg/mL as the internal standard were prepared and processed on each of three consecutive days as outlined in the sections 'Standard Curve Preparation' and 'Sample Preparation'. The concentrations of sparfloxacin were quantified using the calculated calibration curves prepared for each set.

Accuracy was calculated based on the mean percentage error, while precision was evaluated by calculation of intra- and inter-day coefficients of variation (% C.V.)

The mean percentage error was calculated as follows:
Mean percentage error =
[(mean measured concentration – expected concentration)/expected concentration] x 100%
The intra-day and inter-day coefficient of variation was calculated as follows:
C.V. = (SD / mean) X 100

Results and Discussion

Chromatograms

Representative chromatograms of blank rabbit plasma and peritoneal fluid, along with chromatograms of rabbit plasma and peritoneal fluid spiked with sparfloxacin and the internal standard, ciprofloxacin and are shown in **Figure 2.2**. Ciprofloxacin and sparfloxacin appeared as sharp, symmetrical and resolved peaks with retention times of 8 and 16 minutes respectively with a run time of 25 minutes. No interfering peaks resulted from the normal components of rabbit plasma or PDE.

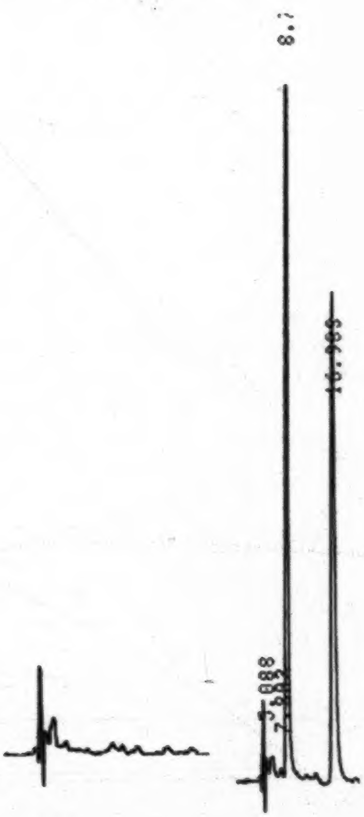


Figure 2.2. Representative chromatograms of blank rabbit plasma (left) and plasma spiked with ciprofloxacin and sparfloxacin. No interfering peaks resulted from the normal components of rabbit plasma. Ciprofloxacin and sparfloxacin appeared as sharp, symmetrical and resolved peaks with retention times of 8.7 and 16.98 minutes respectively with a run time of 25 minutes.

Standard Calibration Curves

A representative calibration curve is shown in **Figure 2.3** A typical regression line through data points would be described by the equation y= 1.4288x + 0.2856 over a concentration range of 0.1- 10 μg/mL.

Linearity was assessed by calculating the correlation coefficient for the concentration range of 0.1-10μg/mL sparfloxacin in plasma and PDF. Standard curves showed excellent linearity between the peak-area ratios of sparfloxacin : internal standard and sparfloxacin concentrations within the examined range of 0.1 – 10 mg/L ($r^2 > 0.99$). The minimum quantifiable concentration was set at 0.2 mg/L

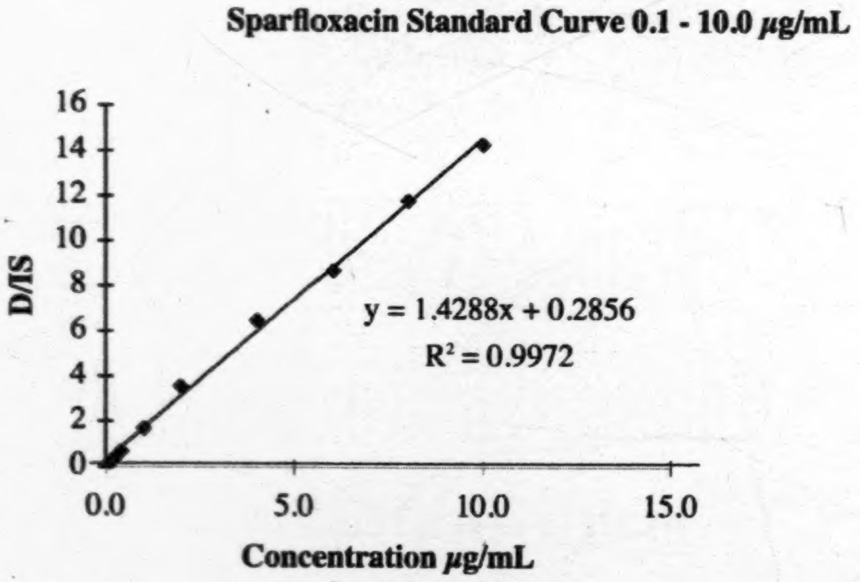


Figure 2.3. **Sparfloxacin Standard Curve.** A typical standard calibration curve could be described by the equation y = 1.4288 + 0.2856. Standard curves showed excellent linearity $r^2 > 0.99$ between the peak area ratio of sparfloxacin :internal standard (D/IS) and spiked sparfloxacin.

Accuracy and Precision

Accuracy of the assay was within 1.67% of the concentration of spiked standards and the intra- and inter-day coefficient of variation (C.V) of the assay ranged from 1.7-13.84 and 9.84--12.94% respectively (**Table 2.2**).

Table 2.2. **Intraday Coefficient of Variation of Sparfloxacin Assay**

Intra-day Variation - Day One

	Day					
Concentration (μg/mL)	**I**	**II**	**III**	**Mean**	**SD**	**%CV**
0.2	0.20	0.19	0.20	0.19	0.00	1.70
4	4.7	4.3	4.33	4.44	0.22	4.99
10	12.17	9.79	10.65	10.87	1.20	11.08

Intra-day Variation - Day Two

Concentration (μg/mL)	**I**	**II**	**III**	**Mean**	**SD**	**%CV**
0.2	0.18	0.20	0.17	0.18	0.01	6.98
4	3.36	4.02	4.3	3.89	0.48	12.40
10	8.8	9.29	11.3	9.80	1.32	13.52

Intra-day Variation - Day Three

Concentration (μg/mL)	**I**	**II**	**III**	**Mean**	**SD**	**%CV**
0.2	0.23	0.23	0.20	0.22	0.01	6.10
4	3.46	4.28	3.87	3.87	0.41	10.59
10	8.13	10.69	9.14	9.32	1.29	13.84

Table 2.3. **Interday Coefficient of Variation and Accuracy.**

			Day 1			Day 2			Day 3					
Concentration (ug/mL)	**No.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Mean**	**SD**	**%CV**	**%Error**
0.2	9	0.196	0.191	0.181	0.178	0.197	0.172	0.225	0.227	0.203	**0.197**	**0.02**	**9.84**	**1.67**
4	9	4.700	4.300	4.300	3.360	4.020	4.300	3.460	4.280	3.870	**4.066**	**0.44**	**10.72**	**-1.64**
10	9	12.170	9.790	10.650	8.850	9.290	11.300	8.130	10.690	9.140	**10.001**	**1.29**	**12.94**	**-0.01**

Table 2.4. Accuracy and Precision of Sparfloxacin Assay

Sparfloxacin Concentration µg/mL	Measured Concentration µg/mL ± SD	Accuracy (%error)	Precision % C.V.
0.2	0.197 ± 0.02	1.67	10.00
0.4	4.07± 0.44	1.64	10.72
10	10.01 ± 1.29	0.01	12.94

PH Dependence of Extraction

Sparfloxacin, (5-amino-1cyclopropyl-6, 8-difluoro-1, 4-dihydro-7-(*cis-3,5-dimethyl-piperazinyl)-4-oxoquinoline-3-carboxylic acid*, is amphoteric.
The extraction of sparfloxacin with ethyl acetate was pH dependent (Table 2.5) due likely to its amphoteric nature (Figure 2.4). An optimal extraction efficacy of 90% was obtained at a pH of 7-8. At pH 2, the extent of extraction was negligible and at pH 10-12, it was less than 50% as shown in Figure 2.5.

Table 2.5. Extraction efficacy in ethyl acetate at varying pH levels

pH	%Extraction	%Extraction	%Extraction	Mean	SD
2	1.2	1.1	1.1	1.1	0.1
3	3.1	2.9	2.7	2.9	0.2
4	4.6	4.2	4.2	4.3	0.3
5	16.9	14.0	15.0	15.3	1.5
6	76.2	67.7	61.5	68.5	7.4
7	102.9	89.2	86.2	92.8	8.9
8	102.7	90.1	85.2	92.6	9.0
9	78.2	82.2	84.4	81.6	3.1
10	54.5	52.0	52.2	52.9	1.4
11	42.0	41.8	43.9	42.6	1.2
12	42.8	40.8	42.2	41.9	1.0
13	50.5	53.8	54.4	52.9	2.1

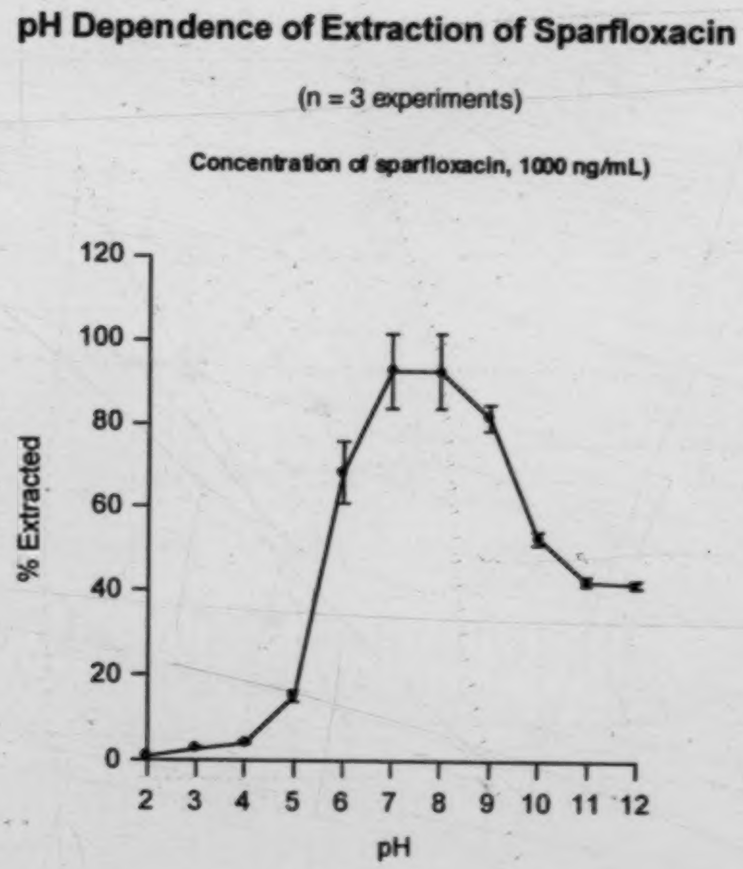


***Figure* 2.4.** Extraction of sparfloxacin with ethyl acetate is pH dependent. An optimal extraction efficacy of 90% was achieved at a pH of 7- 8. At pH 2, the extent of extraction was negligible and at pH 10 – 12 it was less than 50%.

Discussion

In development of this assay, poor extraction efficacy was initially problematic with resultant loss of sensitivity. Extraction with a number of solvents was attempted in an effort to determine the optimal solvent for extraction of sparfloxacin from biological fluids.

When ether was used to extract sparfloxacin, three problems became apparent.

1. Ciprofloxacin, the internal standard, was poorly extracted resulting in small ciprofloxacin (internal standard) peaks.
2. The sensitivity for sparfloxacin was poor.
3. The assay appeared to lose its linearity above 5 or 10 μg/ml, however, this could be improved by splitting the standard curve into two components.

Chloroform and dichloromethane lead to emulsification upon vortex mixing which made separation and complete extraction difficult.

Ethyl acetate provided the best extraction efficacy without the problems encountered with ether, dichloromethane or chloroform , however, sensitivity was lacking.

To circumvent this problem, the possibility of pH-dependency for extraction of sparfloxacin was tested over the range of 2-12 with Sorenson buffer. Adjusting the pH to 7 – 8 resulted in maximal extraction efficacy of 90%. It was concluded that the previously reported difficulty encountered in the quantification of sparfloxacin by HPLC was due to the amphoteric nature of the compound resulting in pH dependent extraction. We chose a pH of 7.4 for extraction.

This assay represented an improvement over the previously published assay (105) that required direct injection of plasma into the HPLC system frequently associated with blocking flow in the HPLC system and damaging the column. Direct injection frequently results in lower sensitivity due to the lack of a concentration process.

Conclusions

The previously reported difficulty encountered in the quantification of sparfloxacin by HPLC assay was due to the amphoteric nature of the compound resulting in pH dependent extraction. Adjusting the pH to 7 or 8 with Sorenson buffer allows for 90% extraction efficacy of sparfloxacin from biological fluids with this assay method. We chose a pH of 7.4 for extraction.

The assay described was sensitive, specific, and linear across the measured concentrations of 0.05 -20 μg/mL.

The assay, developed in rabbit plasma was suitable for the quantification of sparfloxacin in pharmacokinetic studies in the rabbit across the range of 0.05 – 20 μg/mL .

Chapter 3

SYN 1193 Assay

SYN 1193, a new 8-methoxy-fluoroquinolone (Synphar, Edmonton, Alberta, Canada; **Figure 3.1**) is a member of the fluoroquinolone class of antibiotics. SYN 1193 has a broad spectrum of activity, however, it is of particular interest because of its activity against *S. aureus* including methicillin-resistant *S. aureus* (26).

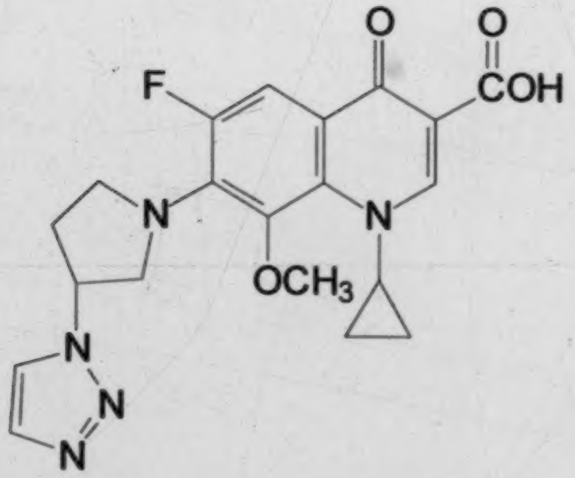


Figure 3.1. Chemical Structure of SYN 1193

No assay for SYN 1193 has been published. Determination of SYN 1193 concentrations in biological fluids had previously proven to be difficult in an assay developed, in house, by Synphar. It was time-consuming since it required a 45 minute period of agitation before extraction. Therefore, a rapid, convenient and sensitive assay that allows for extraction of SYN 1193 from biological fluids was developed.

Materials and Methods

Chemicals (Materials)

SYN 1193 was kindly provided by Synphar, Edmonton, Alberta, Canada. The internal standard, sulindac was obtained from Merck Frosst Canada, Kirkland, Quebec, Canada. Analytical grade acetonitrile was purchased from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada). Orthophosphoric acid was purchased from BDH (Edmonton, Alberta, Canada) analytical grade triethylamine (TEA) was purchased from Mallinckrodt (Paris, KY, USA). Analytical grade ethyl acetate was purchased from BDH (Edmonton, Alberta, Canada). Water was glass double distilled in house. Sorenson phosphate buffer of varying pH was prepared according to a formula outlined in Geigy Scientific Tables (106).

Apparatus and Chromatographic Conditions

The HPLC system consisted of a Waters 6000A Solvent Delivery system (Waters, Mississauga, ON, Canada), Shimadzu SiL-9A Auto–injector (Shimadzu, Tokyo, Japan), a Partisil C8 5μ 250 mm x 4.6 mm reversed-phase analytical column (Phenominex, Torrance, CA, USA), a Nova-Pak C8 guard column (Waters, MA, USA), and a Shimadzu SPD-6AUV Spectrophotometric detector (Shimadzu, Tokyo, Japan). A Model 3390A Hewlett Packard integrator/recorder (Avondale PA USA) was used to determine peak areas. The peak-area ratios of SYN 1193 to sulindac were used to quantify SYN 1193.

The mobile phase was prepared with the addition of 1.75 mL orthophosphoric acid (H_3PO_4) to water to prepare 1 L of orthophosphoric acid 0.025 M, pH 3. Acetonitrile 290 mL and 790 mL 0.025 M orthophosphoric acid were mixed and degassed by filtration with suction through a 0.45 μm membrane filter (Scientific Products & Equipment, Rexdale, Canada). Triethylamine 1.5 mL was added after filtration. The mobile phase was pumped at a flow rate of 1.0 mL/min at ambient temperature. The Shimadzu SPD-6AUV ultraviolet detector (Shimadzu, Tokyo, Japan) was set at 300 nm.

Preparation of Standard Solutions

SYN 1193 Stock Solution

A stock solution of SYN 1193 50 mg/L was prepared with the addition of 5 mg of SYN 1193 to 2.5 mL of 0.1 M NaOH. Sufficient pH 8 Sorenson phosphate buffer was added to produce a final volume of 100 mL. A 1/5 dilution was prepared with the addition of 200 μL stock and 800 μL H_20. A 1/10 dilution was prepared using 100 μL stock and 900 μL H_20 and a 1/100 dilution was prepared using 200 μL of the 1/10 dilution and 1800 μL H_20. The solutions were stored a 4°C.

Internal Standard Stock Solution (Sulindac 0.1 mg/mL)

A stock solution of sulindac 0.1 mg/mL was prepared by dissolving 10 mg of sulindac in 2.5 mL of 0.1 M NaOH. Sufficient pH 8 Sorenson phosphate buffer was added to produce a final volume of 100 mL. A working solution was prepared by diluting 1 mL of the above solution in 4 mL water. The solutions were stored a 4°C.

Sample Preparation

To each 0.5 mL of biological fluid (plasma, peritoneal fluid, or urine), 100 μL of working solution of sulindac was added as the internal standard along with 100 μL

2N HCl and 5mL ethyl acetate. The constituents were extracted with 5 mL of ethyl acetate, vortex-mixed for 1 min, and centrifuged (Adams Dynac centrifuge, Clay-Adams, Parsipanny, NJ, USA) at 1800g for 5 minutes. The organic phase was removed by Pasteur pipette, transferred to fresh numbered tubes and evaporated to dryness without heat (approximately 30 minutes) (Savant Speed Vac Concentrator-evaporator, Emerston Instruments, Scarborough, Canada). The remaining aqueous phase was extracted a second time with 5 mL ethyl acetate, vortex-mixed for 1 minute, centrifuged for 5 minutes, the organic layer removed by Pasteur pipette and added to the residue in the evaporated tubes form the first extraction. The solution was evaporated to dryness without heat for approximately 30 min (Savant Speed Vac Concentrator-evaporator, Emerston Instruments, Scarborough, Canada). The final residue was reconstituted into 200μL mobile phase and 20-100μL of reconstituted sample injected into the system.

Standard Calibration Curve Construction

The SYN 1193 calibration curve was constructed by spiking 0.5 mL aliquots of drug-free rabbit plasma or peritoneal fluid with SYN 1193 to yield concentrations of 0.04, 0.08, 0.2, 0.5, 2.0, 5.0, 8.0, 10.0 and 20 μg/mL as indicated in **Table 3.1**. As an internal standard, 100 μL of working sulindac was added to each sample. The solutions were processed as described previously in 'Sample Preparation'.

Concentration SYN 1193 μg/mL	Volume and Dilution Added
0.0	40 μL of 1/100
0.08	80 μL of 1/100
0.2	200 μL of 1/100
0.5	50 μL of 1/10
2	200 μL of 1/10
5	50 μL of 1/1
8	80 μL of 1/1
10	100 μL of 1/1
20	200μL of 1/1

Table 3.1 **SYN 1193 Standard Calibration Curve**
The SYN 1193 calibration curve was constructed by spiking 0.5 mL aliquots of drug-free rabbit plasma or peritoneal fluid with SYN 1193 to yield concentrations of 0.04, 0.08, 0.2, 0.5, 2.0, 5.0, 8.0, 10 and 20.0 μg/mL. Volume and dilution of SYN 1193 are noted as are the aliquots injected into the system for each concentration.

Assay Validation

To validate the assay, standard curves and tubes spiked with drug of known concentrations, 0.04, 0.08, 5, and 10 μg/mL SYN 1193 were prepared in triplicate. To rabbit blank plasma with 100 μL sulindac working solution added as the internal standard were prepared and processed as outlined in the sections 'Standard Curve Preparation' and 'Sample Preparation' on each of three consecutive days. The concentrations of SYN 1193 were determined using the calculated calibration curves from each set.

Accuracy was calculated based on the mean percentage error, while precision was determined by calculation of intra- and inter-day coefficients of variation (% C.V.) The intra-day and inter-day coefficient of variation was calculated as follows:
% C.V. = (SD/mean) x 100

The mean percentage error was calculated as follows:
Mean percentage error =
[(mean measured concentration – expected concentration)/expected concentration] x 100%

Results and Discussion

Chromatograms

Representative chromatograms of blank rabbit plasma along with those of rabbit plasma and peritoneal fluid spiked with SYN 1193 and the internal standard sulindac are depicted in **Figure 3.2**. SYN 1193 and sulindac appeared as sharp, symmetrical and resolved peaks at 19.24 and 26.6 minutes with a run time of 35 minutes. No interfering peaks occurred from blank rabbit plasma, or PDE.

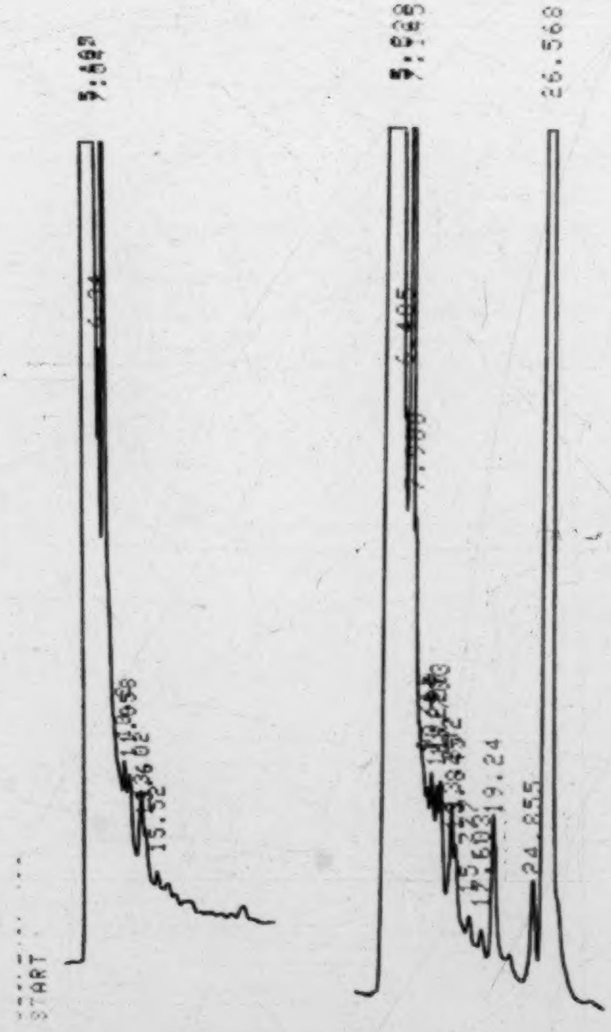


Figure 3.2. Representative Chromatograms

Representative chromatograms with blank rabbit plasma (left) and rabbit plasma showing sharp, symmetrical resolved peaks of SYN 1193 and sulindac (I.S.) with retention times of 19.24 and 26.57 minutes with a run time of 35 minutes.

Standard Curve Construction SYN 1193

The standard curve was constructed by plotting the ratio (D/IS) of the area under the curve of the drug concentration (SYN 1193) (D) to the area under the curve of the concentration of internal standard (IS) measured by HPLC vs. the concentrations added to each tube.

Table 3.2. **SYN 1193 Standard Curve Construction**

Sample	Concentration SYN 1193 ug/ml	D (Area SYN 1193)	IS (Area Sulindac)	D/IS Ratio
1	0	0	0	
3	0.02	23924	1932249	0.012
4	0.04	53302	2130081	0.025
5	0.06	91910	2356532	0.039
6	0.08	135705	2367717	0.057
7	0.1	117765	1583152	0.074
8	0.4	237606	694028	0.342
9	0.8	489301	836657	0.585
10	1	554648	714836	0.776
11	4	958159	285990	3.350
12	10	2221581	278855	7.967
13	20	4462544	284730	15.673

A representative calibration curve is depicted in **Figure 3.3**. The best-fit lines passing through the experimental points could be described by the equation y = 0.77865x – 0.0175 over a range of 0.04 – 20 μg/mL. The minimum quantifiable concentration was set at 0.04 μg/mL.

Linearity was assessed by calculating the correlation coefficient for the concentration range of 0.4 - 20μg/mL in plasma. Standard calibration curves in rabbit plasma showed excellent linearity ($r^2 > 0.99$) between the peak-area ratios of SYN 1193:sulindac (D/IS ratio) and known SYN 1193 concentrations Standard curves were prepared in Dianeal 1.5% and rabbit blank PDE to ensure sensitivity and linearity. Standard calibration curves in Dianeal 1.5% and blank rabbit plasma showed excellent linearity ($r^2 > 0.99$) between the peak-area ratios of SYN 1193:sulindac (D/IS ratio) and known SYN 1193 concentrations. The best-fit line passing through the experimental points over a range of 0.04 – 20 μg/mL in Dianeal 1.5% could be described by the equation y = 0.6697x – 0.0317. In blank rabbit PDE, the best-fit line could be described by the equation y = 0.7558 – 0.0328. Representative calibration curves of SYN 1193 in blank rabbit plasma **(Figure 3.3)** Dianeal 1.5% **(Figure 3.4)** and blank rabbit PDE **(Figure 3.5)** are depicted below.

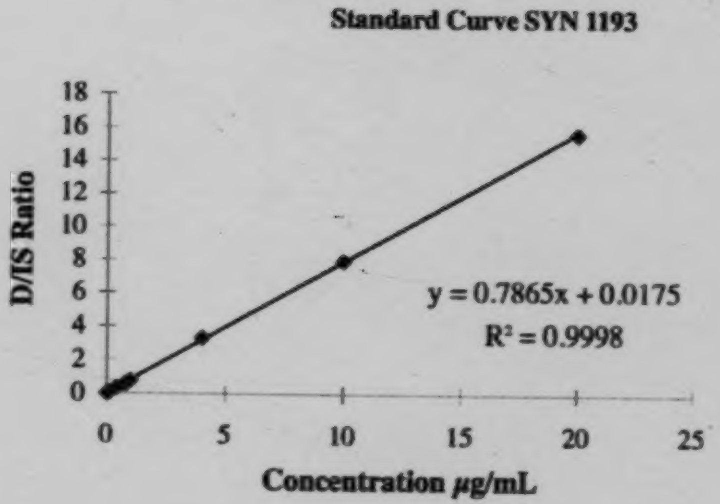


Figure 3.3. **Representative Standard Calibration Curve SYN 1193 in Plasma**
A representative standard calibration curve in rabbit plasma of the relationship between the SYN 1193/IS ratio (D/IS ratio). Corresponding spiked plasma concentrations could be described by the equation y= 0.7865x-0.0175. Standard curves showed excellent linearity $r^2 > 0.99$ between the peak area ratio of SYN 1193: sulindac internal standard (D/IS) and spiked SYN 1193 concentration.

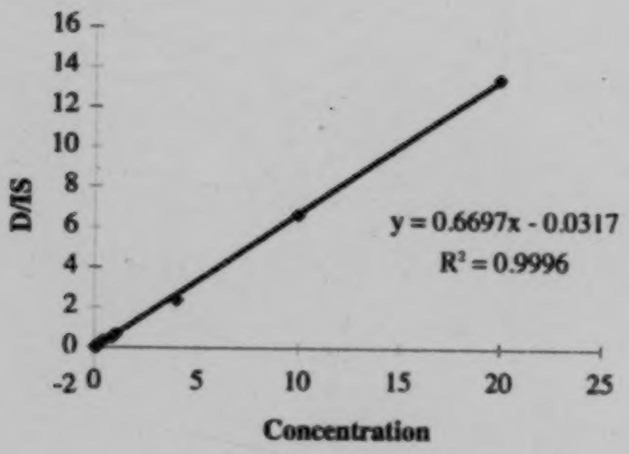


Figure 3.4. **Representative Standard Calibration Curve SYN 1193 in Dianeal 1.5%.** A standard calibration curve in Dianeal 1.5% of the relationship between the SYN 1193/IS ratio (D/IS ratio). Corresponding spiked plasma concentrations could be described by the equation y= 0.6697x - 0.0317. The standard curve showed excellent linearity $r^2 > 0.99$ between the peak area ratio of SYN 1193: sulindac internal standard (D/IS) and spiked SYN 1193 concentration.

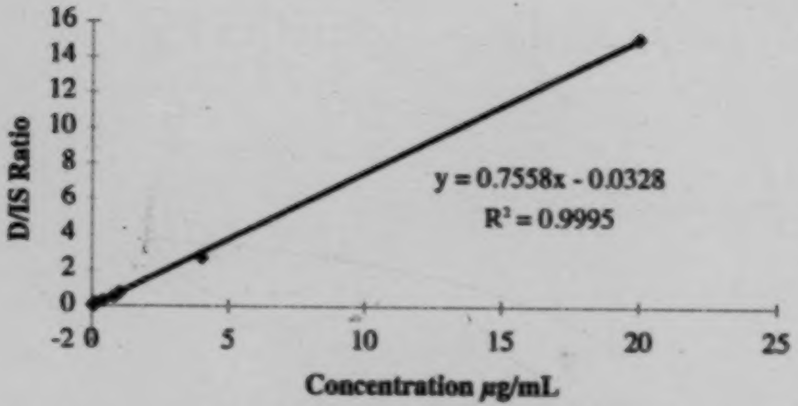


Figure 3.5. **Representative Standard Calibration Curve SYN 1193 in PDE.** A standard calibration curve in PDE of the relationship between the SYN 1193/IS ratio (D/IS ratio). Corresponding spiked PDE concentrations could be described by the equation y= 0.7558x - 0.0328. The standard curve showed excellent linearity $r^2 > 0.99$ between the peak area ratio of SYN 1193: sulindac internal standard (D/IS) and spiked SYN 1193 concentration.

Precision and Accuracy

The intra-day %C.V. ranged from 1.58 – 11.47% **(Table 3.3)** and the inter-day %C.V. ranged from 2- 8% **(Table 3.4)**. Within the examined range of 0.04 – 10 μg/mL, the accuracy of the assay was within 16%.

Table 3.3. **Intra-day Coefficient of Variation and % Error of SYN 1193**

Intra-day Variation - Day One

Concentration (μg/mL)	I	II	III	Mean	SD	%CV	%Error
0.04	0.036	0.036	0.037	0.036	0.001	1.589	9
0.08	0.080	0.069	0.069	0.073	0.006	8.740	9
5	5.520	5.030	5.090	5.213	0.267	5.127	-4
10	10.000	10.620	10.240	10.287	0.313	3.039	-3

Intra-day Variation - Day Two

Concentration (μg/mL)	I	II	III	Mean	SD	%CV	%Error
0.04	0.033	0.041	0.040	0.038	0.004	11.471	5
0.08	0.065	0.072	0.074	0.070	0.005	6.719	12
5	4.900	5.110	4.970	4.993	0.107	2.141	0
10	9.890	10.096	10.290	10.092	0.200	1.982	-1

Intra-day Variation - Day Three

Concentration (μg/mL)	I	II	III	Mean	SD	%CV	%Error
0.04	0.032	0.035	0.034	0.034	0.001	4.478	16
0.08	0.068	0.069	0.066	0.068	0.001	2.095	15
5	5.200	4.866	4.832	4.966	0.203	4.095	1
10	9.880	9.994	10.330	10.068	0.234	2.324	-1

Table 3.4. *SYN 1193 Interday Variation and Accuracy*

	Day (1)			Day (2)			Day (3)						
Conc. (μg/mL)	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**Mean**	**SD**	**%CV**	**% Error**
0.04	0.036	0.036	0.037	0.033	0.041	0.040	0.032	0.035	0.034	0.036	0.003	8	10
0.08	0.080	0.069	0.069	0.065	0.072	0.074	0.068	0.069	0.066	0.070	0.005	6	12
5	5.520	5.030	5.090	4.900	5.110	4.970	5.200	4.866	4.832	5.058	0.212	4	-1
10	10.000	10.620	10.240	9.890	10.096	10.290	9.880	9.994	10.330	10.149	0.243	2	-1

Relative Extraction Efficacy SYN 1193

The relative extraction efficacy of ethyl acetate, chloroform and ether were assessed using spiked samples in rabbit plasma. Based in relative extraction efficacy, chloroform appeared to be the most appropriate solvent for extraction.

Table 3.5. Relative Extraction Efficacy

SYN 1193 Comparison of Extraction Efficacy			
Ethyl acetate Concentration µg/ml	**D**	**IS**	**D/IS**
0.02	0.95	71.5	0.013287
2	52.08	35.84	1.453125
Chloroform Concentration µg/ml	**D**	**IS**	**D/IS**
0.02	0.98	65.51	0.0149901
2	51.79	31.2	1.659936
Ether Concentration µg/ml	**D**	**IS**	**D/IS**
0.02	0.47	74.1339	0.00634
2	45.75	40.64	1.125738

Discussion

Extraction Efficacy

Although the relative extraction efficacy would point to chloroform as the most suitable solvent for extraction of SYN 1193, chloroform extraction resulted in emulsion formation making separation of the organic layer difficult. Ethyl acetate proved to be a superior solvent for the extraction of SYN 1193 without emulsification.

Due to pH dependency of extraction of sparfloxacin and structural similarities between sparfloxacin and SYN 1193, extraction of SYN 1193 was attempted using Sorenson buffer pH 8 to adjust the pH to 7.4. Adjustment of pH to 7.4 for extraction resulted in well-resolved peaks of SYN 1193 and ciprofloxacin. Approximately 7 minutes apart with only 1 minute of vortex-mixing required for each extraction.

The assay described here is sensitive and specific with a sensitivity of 0.04 μg/mL. The MIC of SYN 1193 for *S. aureus* MU7056, the bacterial pathogen to be tested in the proposed animal portion of our studies was 0.5μg/mL. A sensitivity of 0.04 μg/mL would allow concentrations of SYN 1193 to drop 10-fold below the MIC of 0.5μg/mL before reaching the limit of the assay in rabbit plasma, PDE or Dianeal 1.5%.

Conclusions

The assay described was sensitive, specific and suitable for the planned studies of SYN 1193 in treatment of *S. aureus* MU7056 in the rabbit model peritoneal dialysis.

This assay represents an improvement over the assay previously designed in-house by Synphar requiring 45 minutes of mixing with a shaker for each extraction process.

Chapter 4

Comparative Study of SYN 1193, Vancomycin and Placebo in the Treatment of Peritonitis in Rabbits receiving Continuous Peritoneal Dialysis

Methods:

Rabbit Pre-study

Two New Zealand White (NZW) Specific Pathogen Free (SPF) Rabbits weighing 3.0 and 3.06 kg were studied to determine the optimal doses of SYN 1193 and sparfloxacin to be adopted in the test animals. All rabbits used in the pre-study and further studies were subject to quarantine for one week prior to entry into the research protocol.

In preparation for the study, sterile Silastic peritoneal dialysis catheters specifically designed for the project to mimic the Tenckhoff peritoneal catheters used in humans for peritoneal dialysis were inserted as described in the following sections, Preparation for Peritoneal Dialysis Catheter Insertion and Peritoneal Dialysis Catheter Insertion. This pre-study rabbit was not infected with *S. aureus* MU 7056 prior to drug administration and pharmacokinetic studies. Following a 2 day rest period to allow for healing of catheter insertion and exit sites, the animals underwent peritoneal dialysis with 3 exchanges of 200 mL Dianeal 1.5% with dwell times of 4 hours followed by one further exchange of 200 mL with an overnight dwell of 12 hours. This animal received SYN 1193 20 mg/kg i.p. once only with the first dialysis exchange. A set of plasma and peritoneal fluid samples were taken at 0, 0.5, 1, 2, 4, 8, 12 and 24 hr to estimate the pharmacokinetic profile of the SYN 1193 and drug doses required to achieve the pharmacodynamic endpoints in study animals.

The MIC of *S. aureus* MU7056 was determined by Synphar Laboratories by broth dilution following NCCLS standards.

Quarantine

Specific Pathogen Free (SPF) New Zealand White rabbits were obtained from the Charles River Animal Facility. Each group of rabbits was weighed, tagged for proper identification and quarantined for one week prior to the commencement of the 15 day research protocol. The rabbits were fed Purina rabbit chow and given free access to water during the quarantine period and throughout the study protocol.

Preparation for Peritoneal Dialysis Catheter Insertion

Robinul (glycopyrrolate) 0.3mL and buprenorphine 0.3 mL were administered intramuscularly (i.m.) twenty minutes before anesthesia. Each rabbit was shaved from dorsal midline to ventral midline, point of hip to mid-thorax. In addition, the area behind the left ear and between the ears at the base of the cranial-lumbar spine was shaved. The shaved areas were cleaned with 3 surgical scrubs of Betadine and alcohol. The animals were placed in clean cages and transferred to the animal surgical suite for catheter placement.

Peritoneal Dialysis Catheter Insertion

The rabbit was placed on sterile drapes on the operating room table. Halothane was delivered by mask. Corneal reflexes and respiratory rate were monitored visually to ensure adequate anesthesia. The abdominal skin was cleaned with Betadine and covered with surgical drapes. All surgical instruments were sterilized and strict aseptic technique used for all surgical procedures. The sterile Silastic peritoneal dialysis catheters, 32.5 cm in total length with a single 1.5 cm Dacron cuff placed at 5 cm were implanted in the peritoneal cavity and tunneled under the skin to exit at the base of the cranial-lumbar spine according to the protocol designed by Read et al (103). A Zem paramedian incision was made through all layers with a sterile scalpel. A small incision was made in the peritoneum and the proximal end of the catheter was inserted into the peritoneal cavity with the tip pointed towards the pelvic paracolic gutter. The peritoneum and internal fascia were sutured to the proximal portion of the Dacron cuff with continuous sterile 5-0-Vicryl. The external fascia was sutured to the distal portion of the cuff. A small stab incision was made through the skin at the base of the cranial-lumbar spine. Blunt forceps were inserted through this incision and used to form a tunnel by blunt dissection through the subcutaneous tissues to exit at the abdominal incision. With the forceps still in place, the distal end of the catheter was grasped with the forceps and the catheter pulled through the subcutaneous tunnel to exit at the base of the cranial-lumbar spine. The catheter was secured at the exit site with a purse-string suture with 4-0-silk. The incision was closed in layers with continuous 4-0-chromic gut for the subcutaneous tissues and 4-0-silk for the skin. The remaining 1/2 inch catheter was closed with an injection cap which was replaced with a luer–lock valve once dialysis was begun. A specially designed Kevlar jacket with a zipper up the back was placed on the rabbits to ensure that they did not chew at the catheter or the wound site. An Elizabethan collar was placed on the rabbits to protect the catheter from the rabbits. The rabbit model showing exit site of PD catheter is shown in **Figure 4.1.**

Post-operative care

The rabbits were housed individually, fed Purina Rabbit Chow, given free access to water and offered oatmeal when appetites lagged due to the development of peritonitis. They were monitored daily for food and water consumption, urination, defecation and incision site abnormalities. Buprenorphine was administered for pain following surgery and any time if needed during the experiment.

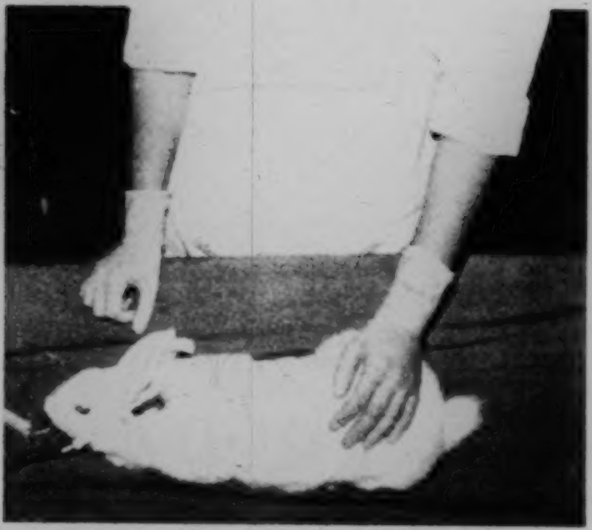


Figure 4.1. Rabbit Model. Rabbit Model showing exit site of PD catheter at the base of cranial-lumbar spine.

Isolation Procedures

Throughout the experiments, caution was taken to ensure isolation of MRSA contaminated articles and samples. The rabbits were housed in a containment facility with restricted access. Persons wishing to enter the facility were required to have special permission, a key for the elevator to the restricted facility and a key for the restricted animal treatment area.

Before entering the restricted animal treatment facility, every person was required to:

1. Change into surgical scrubs and lab coat in the animal facility change room,

2. Access the restricted lower level through a keyed elevator
3. Change lab coats upon entering the lower level.
4. Access the animal treatment facility through a locked change area with restricted access.
5. Change into a new set of surgical scrubs.
6. Put on two pairs of plastic knee-high boots, two pairs of latex gloves and a mask upon moving into the animal treatment facility anteroom before entering the animal treatment room.

Upon exiting the animal treatment room, each person was required to:

1. Remove one pair of gloves and one pair of boots while stepping out of the animal treatment room into the anteroom.
2. Place the discarded boots and gloves in the garbage container in the animal treatment room.
3. Remove the remaining gloves and handwash with Hibitaine just before reentering the change room.
4. Remove the remaining pair plastic boots and step into a solution of bleach before entering the shower anteroom.
5. Remove surgical scrubs in the shower anteroom. All workers had the option of showering if they had been contaminated.
6. Change into the original surgical scrubs and lab coat in the change room before exiting and locking the room.
7. Change lab coats upon reaching the restricted access elevator.
8. Remove the surgical scrubs and wash hands upon returning to the animal facility change room.

All items entering the restricted animal treatment room, including all documentation sheets were autoclaved or disinfected before they were allowed to leave the facility. All animal samples were double-bagged and sprayed with bleach solution before removal from the anteroom. When assaying samples or manipulating samples in the laboratory, all animal samples were processed or manipulated in a biohazard hood.

Administration of Test Drug, Blood and PDE Sampling Pre-test Rabbit

Following a two day rest period, the rabbit was given SYN 1193 20 mg /kg intraperitoneally (i.p.) with 200 mL Dianeal 1.5% at 8:00 hr. Two more exchanges with 4 hour dwell times were carried out at 12:00 noon and 16:00. The final exchange with 200 mL Dianeal was carried out at 20:00 followed by a 12 hour dwell time (see **Figure 4.1**). The drug was administered with only the first of the four exchanges. A complete set of blood and peritoneal fluid samples were taken at 0, 0.5, 1, 3, 6, 9, 12 and 24 hours post drug administration to determine the dose necessary to provide adequate peritoneal and blood concentrations for the study. Blood samples (1 mL) were obtained by marginal ear venipuncture, centrifuged, plasma collected and placed on ice. PDE samples (5mL) were obtained through the PD catheter after discarding the first 3mL of PDE withdrawn to allow for the volume of fluid in the dead space of the catheter. PDE samples were immediately placed on ice. Plasma and PDE samples were frozen at –20°C until assay.

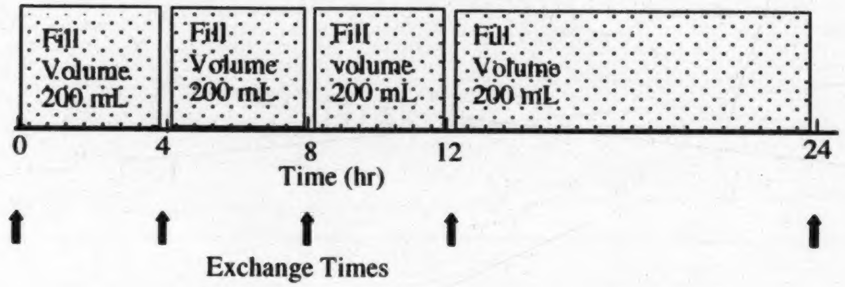


Figure 4.2. **Dialysis Schedule**. Four exchanges of 200mL Dianeal 1.5% were carried out at 0, 4, 8 and 12 hours Days 11 – 14 inclusive. The first three dwell times were 4 hrs each while the final exchange was allowed to remain in the peritoneal cavity overnight for a dwell time of 12 hour.

Results

Pre-study Test Rabbit Receiving SYN 1193

Based on preliminary work by Synphar, we chose a dose of 20 mg/kg to be given i.p. Dialysis exchanges with 200 mL Dianeal were performed 4 and 8 hours post-dose.

Minimum Inhibitory Concentration of SYN 1193 for S. aureus MU7056

The minimum inhibitory concentration of SYN 1193 for *S. aureus* MU7056 was found to be 0.5 μg/mL by broth dilution performed by Synphar Laboratories following standard NCCLS guidelines.

Plasma and Peritoneal Fluid Concentrations of SYN 1193 in Pre- test Rabbit

Four exchanges with 200 mL Dianeal 1.5% were performed out at 08:00, 12:00, 16:00 and 20:00 hr resulting in three 4 hr dwell times and a 12 hr overnight dwell. SYN 1193 20 mg/kg was administered i.p. once only with the first fill of 200 mL of Dianeal 1.5% at 8:00 hr. A complete set of blood and PDE concentrations were taken at 0, 0.5, 1, 3, 6, 9, 12 and 24 hr. Blood was centrifuged and plasma decanted. Samples were analyzed by HPLC as described in Chapter 3, SYN 1193 Assay. Due to difficulties with marginal ear vein puncture, blood samples obtained at 0.5, 2, 4 and 24 hrs were of insufficient volume for analysis. An attempt had been made to insert a catheter into the marginal ear vein to facilitate multiple sampling, but the catheter failed.

Plasma concentrations of SYN 1193 in the test rabbit were 29.9 μg/mL at 1.3 hrs but were negligible at 9 and 12 hrs post drug administration. Plasma concentrations are reported in **Table 4.1.**

Table 4.1. **Plasma Concentrations SYN 1193 – Pre-test Rabbit 1**
Plasma concentrations of SYN 1193 for Pre-test Rabbit 1 following one dose of SYN 1193 20 mg/kg i.p. in the first of 4 dialysis exchanges at 0, 4, 8 and 12 hours over 24 hours.

Time (hr)	**Plasma Concentration *(μg/mL)**
0	**0.00**
0.5	insufficient volume
1.3	**29.9**
9	**0.00**
12	**0.06**
24	insufficient volume

PDE Samples in SYN 1193 Pre-test Rabbit

Peritoneal dialysis effluent (PDE) samples were withdrawn from the indwelling peritoneal catheter by syringe 1, 3, 6, 9, 12 and 24 hours after dosing. An extra sample was retained at 8 hr at the time of dialysis exchange. Drug concentrations were analyzed by HPLC assay developed as previously described in Chapter 3, SYN 1193 assay. Maximum PDE SYN 1193 concentrations of 83.5 μg/mL were attained in the test rabbit at 1 hour with trough concentrations of 1.4 and 1.2 at 12 and 24 hours. PDE concentrations were maintained above the MIC of *S. aureus* MU7056 for the entire 24 hr dosing interval. PDE concentrations are reported in **Table 4.2.**

Table 4.2. **PDE Concentrations SYN 1193 Pre-test Rabbit**
PDE concentrations of SYN 1193 in Pre-test Rabbit 1 following SYN 1193 20 mg/kg i.p. with the first of four dialysis exchanges only. PDE concentrations were maintained above the MIC of *S. aureus* MU7056 of 0.5 μg/mL for the entire 24 hr dosing interval.

Time (hr)	PDE Concentration (μg/mL)
1	83.5
3	48.2
6	5.6
8	3.4
10	3.7
12	1.4
24	1.2
blank	0

Peak/MIC Ratios of SYN 1193 Achieved in Plasma and PDE

Based on an MIC of 0.5 μg/mL, the peak/MIC ratios in plasma and PDE of pre-test rabbit 1 were 59.8 in plasma and 167 in PDE as shown in **Table 4.3**.

Table 4.3. Peak concentrations of SYN 1193 measured in plasma and PDE and Peak/MIC ratios of SYN 1193 achieved in plasma and PDE with pre-test rabbit 1.

Site	Peak Concentration μg/mL	MIC μg/mL	Peak /MIC Ratio
Plasma	29.9	0.5	59.8
PDE	83.5	0.5	167.0

AUC_{24}/MIC Ratios of SYN 1193 Achieved in Plasma and PDE

Based on an MIC of 0.5 μg/mL, the AUC_{24}/ MIC ratios in plasma and PDE of pre-test rabbit receiving 20 mg/kg SYN 1193 once only in the first of four dialysis treatments were 270 in plasma and 1373 in PDE as shown in **Table 4.4.**

Table 4.4. AUC SYN 1193 measured in plasma and PDE and AUC_{24}/MIC ratios of SYN 1193 achieved in plasma and PDE with pre-test rabbit 1 with an MIC of 0.06 for *S. aureus* MU7056.

Site	AUC SYN 1193 hr^{-1}	MIC μg/mL	AUC_{24}/MIC Ratio
Plasma	135	0.5	270
PDE	687	0.5	1373

Discussion Pre-test Rabbit Study of SYN 1193

The goal of the studies in the pre-test rabbit was to determine the dose of SYN 1193 to be administered i.p. in order to attain adequate concentrations in blood and PDE throughout the 24 hour dosing interval despite continued dialysis exchanges at 4, 8 and 12 hours without further administration of SYN 1193 with these exchanges.

In this preliminary study, the peak concentrations achieved by administration of SYN 1193 20mg/kg administered i.p. once daily with only the first of 4 exchanges of 200 mL Dianeal were 29.9 μg/mL and 83.5 mg/mL in plasma and PDE respectively. Trough concentrations in plasma were 0 at 9 hr and 0.06 at 12 hr while trough concentrations were maintained at 1.4 and 1.2 at 12 and 24 hours in PDE.

The fluoroquinolone class of antibiotics exhibits concentration-dependent pharmacodynamics. Successful treatment of infection correlates best with peak concentrations of the antibiotic equivalent to at least 10 – 20 times the MIC of the infecting bacterial pathogen, and an AUC$_{24}$/MIC ratio of 60-80 for *S. aureus*. The fluoroquinolone antibiotics also demonstrate a post-antibiotic effect of 1.5 -2.5 hr(35). Clinical studies have shown that the possibility of resistance is reduced if the peak/MIC ratio is greater than 8 – 10 and the AUC /MIC ratio is $\geq$ 100.

The MIC of SYN 1193 for *S. aureus* MU7056 was determined to be 0.5 μg/mL by Synphar through standard broth dilution following NCCLS guidelines. In this preliminary study, the resulting peak/MIC ratios for SYN 1193 of 59.8 in plasma and 167.0 in PDE exceed the target range for the treatment of gram-positive infections in plasma and PDE. The plasma sample at 0.5 hr was of insufficient volume to analyze and there was some concern that the true peak had been missed.

PDE concentrations of SYN 1193 were maintained above the MIC of *S. aureus* MU7056 of 0.5 μg/mL the entire 24 hr dosing interval. However, plasma concentrations dropped to 0 by 9 hours. Even considering the post-antibiotic effect, there was concern that this would not be sufficient to maintain adequate antibiotic concentrations throughout the 24 hour dosing interval. Although the literature reports that the main pharmacodynamic parameters associated with successful treatment are the Peak / MIC ratio and AUC / MIC ratio, these studies were not been done under conditions of peritoneal dialysis where the drug is removed from the peritoneal cavity four times a day so that drug concentrations may drop to concentrations below the MIC for prolonged intervals. On this basis, it was decided that the dose of SYN 1193 must be changed to 20 mg/kg b.i.d. to ensure maintenance of plasma concentrations.

AUC_{24}/MIC Ratios of SYN 1193 Achieved in Plasma and PDE

The 24 hr AUC of SYN 1193 was 135 h^{-1} in plasma and 1372.1 h^{-1} in PDE (**Table 4.5**). Resulting AUC_{24}/MIC ratios with an MIC of SYN 1193 for *S. aureus* MU7056 of 0.5 μg/mL were 270 and 1373 in plasma and PDE respectively, well in excess of the targets of 50-80 correlated with efficacy in the treatment of *S. aureus* or the AUC/MIC ratio of >100 correlated with the prevention of the development of resistance.

Table 4.5. AUC_{24} SYN 1193 in plasma and PDE and AUC_{24}/MIC ratios of SYN 1193 achieved in plasma and PDE with pre-test rabbit 1.

Site	**AUC SYN 1193 hr^{-1}**	**MIC μg/mL**	**AUC_{24}/MIC Ratio**
Plasma	135	0.5	270
PDE	687	0.5	1373

Study Animals Receiving SYN 1193, Vancomycin or Placebo - Protocol

Twelve (3 groups of 4) New Zealand White (NZW) Specific Pathogen Free (SPF) Rabbits weighing 3-3.5 Kg were studied. Each rabbit in each group of rabbits (n=4) had a peritoneal catheter implanted, was inoculated with a human strain of MRSA MU7056 (obtained from the Provincial Laboratory of Alberta isolates from CAPD patients with peritonitis), dialyzed and treated as follows. Group 1 (n=4), the control group, received placebo, group 2 (n=4) received SYN 1193 20 mg/kg i.p. b.i.d. and group 3 (n=4) vancomycin 20 mg/kg i.p. b.i.d. Due to the intensity of the experiments, and the requirement for containment due to MRSA, only 1 group of 4 rabbits could be studied during each 15-day period. Each group of rabbits were weighed, tagged and quarantined for one week prior to the commencement of a 15 day research protocol as follows:

Day 1 (Friday)	Placement of a peritoneal dialysis catheter in the peritoneal cavity of the rabbit.
Days 2-3	No interventions. Time to allow healing of the incision site and catheter exit site.
Days 4-7	Inoculation of the of the catheter exit site with methicillin-resistant *Staphylococcus aureus* MU 7056 and culture of the fluid in peritoneal cavity daily to ensure the development of peritonitis. Sterile Dianeal 1.5% 10 mL was injected each morning to ensure fluid for withdrawal for culture. (Following response in Control group inoculation was deferred until Day 5). Once cultures of PD fluid were positive, no further inoculation was carried for that rabbit.
Day 8 (Friday)	Further inoculation of catheter exit site with *MRSA* MU7056 only in rabbits with negative cultures Day 7.
Day 10 (Sunday)	Two dialysis exchanges with 200 mL Dianeal 1.5% (morning and evening) to ensure the development of peritonitis.

Days 11- 14	Dialysis with 200 mL Dianeal 1.5% 4 times a day at 0:800, 12:00, 16:00 and 20:00 hr with the administration of the test drug b.i.d. at 08:00 and 20:00 hr. Blood and peritoneal fluid sampled on Days 11 and 14.
Day 15	Animals sacrificed, necropsy performed, tissue and catheter samples obtained and processed.

The need for isolation of the study due to working with methicillin-resistant *S. aureus* as well as the intensity of the study requiring q.i.d. dialysis of the rabbits and multiple samples of plasma and PDE limited the number of rabbits to four in each group.

Quarantine, pre-operative treatment and catheter placement (day 1) were carried out as previously described in the rabbit pre-study test section.

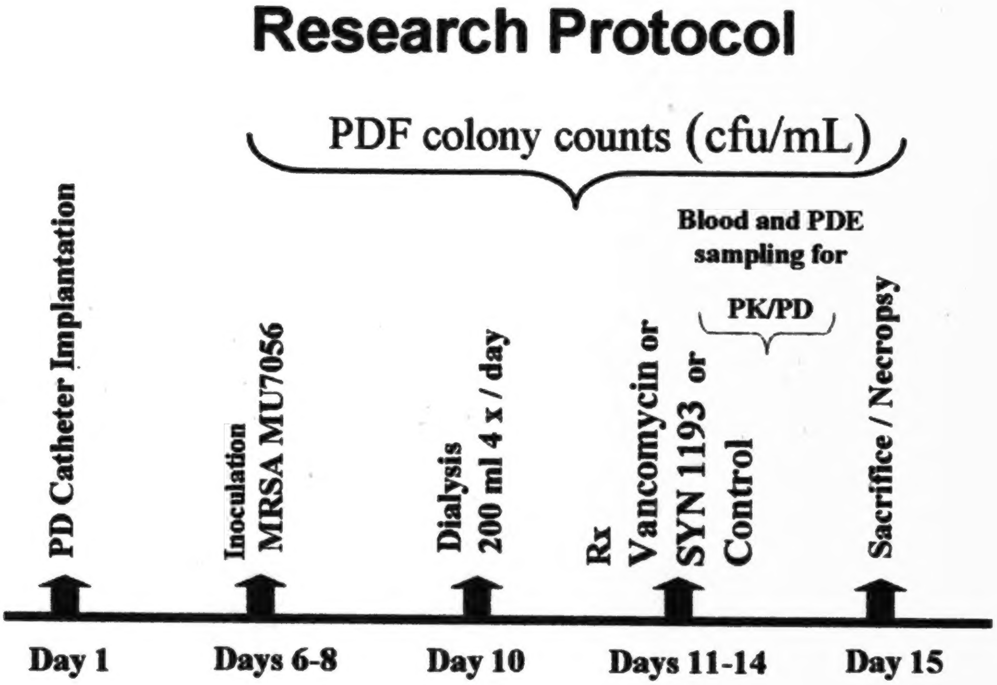

Research Protocol
PDF colony counts (cfu/mL)
PD Catheter Implantation
Inoculation
MRSA MU7056
Dialysis
200 ml 4 x / day
Rx
Vancomycin or
SYN 1193 or
Control
Blood and PDE
sampling for
PK/PD
Sacrifice / Necropsy
Day 1
Days 6-8
Day 10
Days 11-14
Day 15

Figure 4.3. Research Protocol

Inoculation and Induction of Peritonitis

Following a 2-day rest post-operatively to allow for wound healing, peritonitis was induced by inoculation of the catheter exit site with *MRSA* (MU7056) days (Days 4, 6 and 7) until daily peritoneal cultures became positive. The inoculum, 10^4 freshly cultured methicillin-resistant *S. aureus* MU7056 was suspended in 1.0 mL Phosphate Buffered Saline (PBS) and gently introduced between the catheter and the subcutaneous tunnel at the exit site using the syringe tip of a 1 ml syringe without an attached needle. Care was taken not to inject the MRSA MU 7056 into the skin or surrounding tissues. Samples of peritoneal fluid were obtained each morning by injection and withdrawal of 10 mL Dianeal 1.5% through the peritoneal catheter. Day 8, inoculation was repeated in any rabbits culture-negative cultures from Day 7. Day 10 (Sunday), the rabbits were dialyzed with 2 exchanges of warmed Dianeal 200mL to aid in the development of peritonitis[1]. PDE samples were collected each morning, plated on Mueller-Hinton agar. Colony counts were performed before inoculation the following day throughout the inoculation period to verify the development of peritonitis. Once PDE was culture-positive, infected animals were not further inoculated.

Control rabbits were quite ill Day 11, the day treatment would commence in the SYN 1193 and vancomycin treatment groups. One control rabbit needed to be euthanised on compassionate grounds on Day 12 and rest on Day 13. Following this experience, we delayed inoculation in the SYN 1193 and Vancomycin groups until Day 6.

Drug Administration and Pharmacokinetic Studies

Dialysis and Test Drug Administration

Day 11, after confirming the development of peritonitis, the rabbits were dialyzed with 200 mL sterile Dianeal 1.5% for a total of four exchanges each day at 08:00, 12:00, 16:00 and 20:00 hrs on Days 11-14. Dwell times throughout the day were 4 hours, however, the last exchange at 20:00 hr remained in the peritoneal cavity overnight until the next day's dialysis was performed at 08:00 hr for a 12 hour dwell time. Dianeal 1.5% was warmed and slowly administered and removed at the end of each dwell using a sterile 50 mL syringe to perform the exchanges. Strict aseptic technique was observed to avoid any touch contamination during exchange of dialysis solutions. Once dialysis commenced, the PD catheter was flushed with 2.5 mL of heparin 500u/mL daily following the 20:00 hr exchange to prevent catheter blockage. The test drug (vancomycin, SYN 1193 or placebo) was administered with the dialysis fluid b.i.d. at 8:00 and 20:00 hr on Days 11-14 with dialysis exchanges with 200 mL Dianeal 1.5% at 0:800, 12:00, 16:00 and 20:00 hr. Blood, PDF and urine samples were obtained Day 14 (after 4 days of antibiotic treatment) to determine the pharmacokinetic/pharmacodynamic profile of each drug.

Blood Sampling

A complete set of blood samples (1 mL) were collected at 0, 0.5, 1, 3, 6, 9, 12, and 24 hours post 08:00 hr drug administration on day 14 for determination of drug plasma concentrations. Blood was collected by marginal ear venipuncture, centrifuged, plasma collected and placed immediately on ice. Plasma samples were stored in polypropylene tubes at –20° C until assay. Samples from the SYN 1193 group were assayed using the HPLC assay developed as described in Chapter 3. Samples from the vancomycin group were assayed by the University of Alberta

Hospitals Laboratory using a Particle Enhanced Turbidometric Inhibition Immunoassay (PETINIA).

PDE Sampling

PDE samples (5mL) were obtained by syringe through the PD catheter. The first 3 mL of PDE withdrawn were discarded to compensate for the volume of fluid in the dead space of the catheter. PDE samples for determination of drug concentrations were collected immediately before the 08:00 hr drug administration and at 0.5, 1, and 2 hours post drug administration. Extra PDE samples were taken immediately pre-dialysis and post-exchange at 4, 8 and 12 and 24 hours. PDE samples were centrifuged, decanted and immediately placed on ice. PDE samples were frozen at -20°C until assay.

Urine Collection

On day 14 the rabbits were placed in metabolic cages for 24 hours to facilitate urine collection for determination of cumulative drug excretion in urine. Urine was collected every 12 hours. The total volume was measured and recorded. A 5 mL aliquot was taken, placed on ice and frozen at –20°C until assay.

PDE Samples for Culture

PDE samples were collected for bacteriologic culture Days 11-15 before the 08:00 hr dialysis exchange. In cases where rabbits resorbed all of the PDF fluid overnight, 10 mL of Dianeal 1.5% was injected into the peritoneal cavity and withdrawn to obtain a sample for culture.

Sacrifice and Necropsy

Day 15, a 5 mL sample of PDE was taken for culture, rabbits sacrificed and necropsies performed. The rabbits were euthanised by pentobarbital intravenous injection into the marginal ear vein. Necropsy was performed in the animal facility operating theatre.

Necropsy

The rabbit was shaved from the dorsal midline to the ventral midline, point of hip to the mid-thorax. In addition, the area behind the left ear and between the ears at the base of the cranial-lumbar spine and the area following the catheter tunnel was shaved. The shaved area was prepped with 3 surgical scrubs of Betadine and alcohol. A ventral midline incision was made from sternum to pubis through the skin and underlying abdominal muscles. Left and right lateral incisions were made at the level of the diaphragm and across the pubis using a sterile scalpel and forceps. The skin and abdominal muscles were peeled back to expose the abdominal cavity.

The following samples were collected for quantitative bacteriologic studies, taxonomic identification and electron microscopy (TEM, and SEM).

Samples for Quantitative Bacteriologic Studies and Taxonomic Identification

PD Catheter Samples collected for quantitative bacteriologic studies and taxonomic identification included: a 2 cm sample of PD catheter from the peritoneal cavity (Internal catheter), a 2 cm sample of PD catheter from the subcutaneous tunnel (External catheter) and the Dacron cuff.

Sample Processing for Quantitative Bacteriologic Studies and Taxonomic Identification

Interior and external surfaces of PD catheters (2cm) were scraped aseptically using sterile scalpel blades. The blade and scrapings were placed in Mueller-Hinton Broth, vortex mixed, sonicated, plated in serial dilutions on Mueller-Hinton agar (MHA) plates, and incubated 72 hours to determine colony forming units/mL (CFU/mL). Taxonomic identification of recovered organisms was done by the University of Alberta Hospitals Provincial Laboratory. To confirm that *S. aureus* isolated was the same as the infecting strain (MU7056), samples were sent for taxonomic identification to the University of Alberta Hospital Provincial Laboratory along with a sample of the infecting strain, *S. aureus* MU 7056.

Tissue Samples for Bacterial Culture

Tissue samples taken for microbiology included:
Peritoneal wall (duplicate samples 1 cm^2)
Subcutaneous tunnel at 10 cm and 20 cm (duplicate samples 1 cm^2)

Sample Processing of Tissues

Tissue samples were weighed and homogenized using a tissue homogenizer. Samples were vortex mixed, sonicated, plated with serial dilution on Mueller Hinton agar plates in a biological flow hood, and incubated at 37°C for 48 hr to determine CFU/mL/g.

Scanning Electron Microscopy

Samples of the CAPD catheter were taken from control and test groups and prepared for scanning electron microscopy (S.E.M.) by fixation in 2% glutaraldehyde in 0.1 M cacodylic acid buffer (pH 7.4), dehydration through a graded ethanol series, and sputter coating with gold. S.E.M. was done using a Hitachi S450 scanning electron microscope.

Evaluation of Results

Efficacy was determined by comparing bacterial culture, and electron microscopy of PDE, catheter sites, catheter tunnel sites and peritoneal tissue between the study groups. The Kruskall Wallis Test and Dunn's Post-hoc Analysis were used for statistical analysis.

Results

Control Animals – Rabbits 1- 4

Peritoneal Effluent Cultures for Control Rabbits 1 – 4

Control rabbits, 1- 4, were inoculated with *S. aureus* MU7056 days 4, 5, and 6. All 4 rabbits were dialysed twice on day 10 to ensure development of peritonitis. No treatment was begun Day 11, but peritoneal dialysis q.i.d. was continued daily as previously described.

PDE showed growth Day 6 in all control rabbits. Rabbit # 2 became extremely ill by Day 11, after only the first two dialysis treatments Day 10, and was euthanised on compassionate grounds. By day 11, Rabbits 1, 3, and 4 refused to eat or drink and became increasingly ill as indicted by lethargy, posturing, stretching and abnormally formed fecal material. The PD catheters became blocked upon attempts to withdraw PDE in Rabbits 1, 3 and 4 by day 11. On Day 13, after only 2 days on the q.i.d. dialysis protocol, the animals were euthanised on compassionate grounds. Necropsy was performed and tissue samples collected. At necropsy, all rabbits had very distended large and small intestines containing large amounts of air and little if any fecal contents. At least 200 - 250 mL of fluid remained in the peritoneal cavity at necropsy. In addition, fibrinous and adhered peritoneal tissue was observed throughout the abdomen. Catheter malfunction was attributed to blockage by a combination of distended loops of bowel and fibrin/sclerosed peritoneal tissue All control rabbits showed positive bacterial growth in PDE by day 6 and at necropsy (Day 11 for Rabbit 2, and Day 13 for Rabbits 1, 3 and 4). Bacterial growth in PDE ranged from 4.6 x 10^3 to 8 x 10^6 CFU/mL at necropsy.

The experience with this group of control rabbits and concerns about the rapidity and aggressiveness of the induced peritonitis with this human strain of *S. aureus* prompted us to delay the initiation of inoculation with *S. aureus* MU7056 until day 6 in all following groups of rabbits to be continued until PDE cultures were positive. This would be followed by two exchanges of dialysate Day 10 to ensure development of peritonitis and initiation of antibiotic treatment with q.i.d. dialysis Day 11.

PDE Cultures Control Rabbits

Results of PDE cultures, shown in **Table 4.6**, confirm that all control rabbits were infected by Day 6 with colony counts ranging form 2×10^1 CFU/mL to those that were too numerous to count (TNTC). On Day 11, Rabbit 2 was euthanised on compassionate grounds. By Day 11, PD catheters in Rabbits 1, 3 and 4 were blocked to withdrawal of fluids and samples could not be obtained for culture. Rabbits 1, 3 and 4 were euthanised Day 13 on compassionate grounds.

PDE in all control rabbits was infected at necropsy with colony counts ranging from 4.6×10^3 to 8×10^6 CFU/mL. Taxonomic identification of bacterial strains isolated identified coliforms, *S. aureus* MU7056 (S. A. MU7056), and CNS (coagulase negative staphylococci).

***Table 4.6.* PDE Cultures - Control Rabbits.** PDE cultures were positive in all control rabbits by Day 6. PDE cultures were positive in all control rabbits at necropsy with colony counts ranging from 4.6 x 10^3 to 8 x 10^6 CFU / mL. Bacterial strains isolated were identified as *S. aureus* MU7056 (S. A. MU7056). Growth of other contaminants as coliforms, and coagulase negative staphylococci (CNS) were also noted as contaminants.

Growth of *S. aureus* MU7056

	Rabbit # 1 CFU / mL	Rabbit # 2 CFU / mL	Rabbit # 3 CFU / mL	Rabbit # 4 CFU / mL
Day 06	2 x 10^1	3.2 x 10^5	TNTC	TNTC
Day 07	5.6 x 10^5	1.1 x 10^6	1.6 x 10^8	3.3 x 10^6
Day 11	No Sample	8 x 10^6 * CNS	No Sample	No Sample
Day 12	No Sample		6 x 10^6	1 x 10^6
Day 13*	3.1 x 10^7 * Coliforms		4.6 x 10^3 * Coliforms	4.5 x 10^5 * Coliforms CNS

PDE samples for all animals were obtained at necropsy as indicated by *

Tissue and PD Catheter Sample Culture Results – Control Rabbits 1- 4

All rabbits showed physical signs of severe infection. Rabbit 2 was euthanised on Day 10 and Rabbits 1, 3 and 4 on Day 13 on compassionate grounds.

All sites sampled (Dacron cuff, internal catheter, external catheter, peritoneal wall tissue, and the subcutaneous tunnel at 2 sites) showed bacterial growth with the exception of 3 sites in Rabbit 2 **(Table 4.7)**. Many sites showed infection with more than one pathogen in addition to *S. aureus* MU7056. Bacterial strains isolated were identified as *S. aureus* MU7056 (S. A. MU7056), coliforms, and coagulase negative *S. aureus* (CNS).

Table 4.7. Tissue and PD Catheter Sample Culture Results – Control Rabbits
Tissue and PD catheter sample culture results (CFU/g/mL) are correlated with bacterial identification at necropsy in untreated rabbits (Table X). Bacterial strains isolated were identified predominantly as *S. aureus* MU7056 (S. A. MU7056), with occasional growth of coliforms, and coagulase negative *S. aureus (CNS)* as contaminants as noted. Rabbit 2 was euthanised on Day 10 of the protocol and Rabbits 1, 3 and 4 on Day 13 on compassionate grounds.

	Rabbit # 1	**Rabbit # 2**	**Rabbit # 3**	**Rabbit # 4**
Dacron Cuff	1.2×10^{7} Coliforms	2.1×10^{7} CNS	2.9×10^{7} Coliforms CNS	5.0×10^{7} Coliforms
Internal Catheter	2.3×10^{7} Coliforms	1.8×10^{5} CNS	8.4×10^{4} Coliforms	7.0×10^{7} Coliforms CNS
External Catheter	8.1×10^{5} Coliforms	4.0×10^{5}	7.1×10^{7} Coliforms	1.6×10^{6} Coliforms CNS
Peritoneal Wall Tissue	1.4×10^{4} Coliforms	No growth	6.3×10^{3} Coliforms	1.7×10^{3} Coliforms
Peritoneal Wall Tissue	8.1×10^{3}	No growth	1.9×10^{4} Coliforms	1.3×10^{2} Coliforms
Subcutaneous Tunnel 10 cm	9.2×10^{4} Coliforms	2.7×10^{3}	7.2×10^{6} Coliforms CNS	1.6×10^{4} Coliforms
Subcutaneous Tunnel 20 cm	4.3×10^{5} Coliforms	No growth	1.1×10^{4} Coliforms	9.1×10^{4} Coliforms

Scanning Electron Microscopy of Internal and External Catheter Samples

Scanning electron microscopy (SEM) of samples of the internal and external catheter was performed to determine the presence of bacterial biofilm adherent to the catheter samples taken inside the peritoneal cavity (internal catheter) and along the subcutaneous tunnel (exterior catheter). On culture, all catheter samples showed positive growth predominantly with *S. aureus* MU7056 with occasional contaminants such as coagulase negative staphylococci and coliforms. The S.E.M. of adjacent samples of the catheter was positive in all but one sample.

Results of electron microscopy revealed a good correlation with bacterial identification in rabbits 1 and 3 where cocci and rods were seen on electron microscopy and *S. aureus* MU7056 and coliforms were identified at the site. The S.E.M. scan of the external catheter in Rabbit 2 was positive for cocci and rods and the culture showed positive growth. The one site where the S.E.M. scan did not correlate with the culture was the external catheter. At this site, the S.E.M. scan was negative but the culture of an adjacent piece of catheter showed a bacterial count of 1×10^5 organisms with positive identification of coagulase negative staphylococci. This could be attributed to the patchy development of biofilm.

Results of S.E.M. scans are shown in **Table 4.8.**

***Table 4.8. Electron Microscopy and Cultures (CFU/g/mL) of Internal and External Catheter Samples – Control* Rabbits.** Samples from the internal and external catheter showed bacterial growth consisting of predominantly *S. aureus* MU7056 with coliforms and CNS as occasional contaminants as noted.

	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4
S.E.M.				
Internal Catheter (Peritoneal Cavity)	cocci	negative	rods	rods
Culture	2.3×10^{7}	1.8×10^{5}	8.4×10^{4}	7.0×10^{7}
Internal Catheter (Peritoneal Cavity)	Coliforms	CNS	Coliforms	Coliforms CNS
S.E.M.				
External Catheter (Tunnel)	cocci rods	cocci, rods	cocci, rods	cocci
Culture	8.1×10^{5}	4.0×10^{5}	7.1×10^{7}	1.6×10^{6}
External Catheter (Tunnel)	Coliforms		Coliforms	Coliforms CNS

CNS = coagulase negative staphylococci

Study Test Animals Receiving SYN 1193 (Rabbits 9 – 12)

Four New Zealand White rabbits received SYN 1193 20 mg b.i.d. i.p. at 08:00 and 20:00 hr Day 11. Four exchanges of peritoneal dialysis were carried out at 08:00, 12:00, 16:00 and 20:00 hr each day with three 4 hour dwell times and a 12 hour overnight dwell (Days 11-14). A complete set of blood samples were taken at 0, 0.5, 1, 2, 4, 8, and 12 hr Day 14. PDE concentrations were taken at 0, 0.5, 1 and 2 hr, 4 and 8 hr pre- and post-dialysis exchanges and 12 hr day 4 of treatment with SYN 1193 (Day 14 of the protocol). Blood was centrifuged and plasma decanted. Samples were analyzed by HPLC as described in Chapter 2, SYN 1193 Assay.

Due to malfunction of the PD catheter in Rabbit 12, we were unable to withdraw any fluid from Day 10 onwards and the rabbit received dialysis fluid and SYN 1193 twice daily at 08:00 and 20:00 hr but no fluid was withdrawn.

Peritoneal Effluent Cultures

Rabbits 9 – 12 Pre- and Post-Treatment with SYN 1193

Rabbits 9-12 were inoculated with *S. aureus* MU7056 days 6 and 7 and dialysed with 200 mL Dianeal 1.5% twice on Day 10 at 08:00 and 20:00 to ensure development of peritonitis. Day 11, treatment with SYN 1193 20 mg/kg i.p. b.i.d. was initiated and peritoneal dialysis continued q.i.d. as previously described. SYN 1193 treatment was continued to the end of Day 14.

Bacterial growth was detected by Day 8 in the PDE of Rabbit 12 and all rabbits by Day 11 with colony counts ranging from 2×10^4 to 1×10^6 CFU/mL Day 11 before initiation of treatment with SYN 1193 **(Table 4.9)**.

From Day 9 onwards, the catheter malfunctioned in Rabbit 12. Fluid could only be administered through the PD catheter and not withdrawn. During this time, Rabbit 12 received dialysis fluid and antibiotic b.i.d., but no fluid was withdrawn and further dialysis as per protocol was not performed. It is interesting to note that Rabbit 12 was the first to show a positive culture (Day 08) and had the highest colony count Day 11 of 1 x 10^6 CFU/mL which may have contributed to catheter malfunction.

By Day 12, after 1 day of antibiotic treatment and dialysis q.i.d., Rabbits 9, 10 and 11 became very ill, however by Day 13, after 2 days of antibiotic treatment and dialysis q.i.d., the rabbits improved significantly.

On Day 15, following 4 days of treatment, the rabbits were euthanised and necropsy performed as per protocol. No growth was detected in the PDE of Rabbits 9 and 10. Rabbit 11 showed bacterial growth of 1.3 x 10^2 CFU/mL. No sample was available for Rabbit 12 due to catheter blockage.

Table 4.9. PDE Cultures Pre and Post Treatment with SYN 1193.

PDE cultures (CFU/mL) pre and post-treatment with SYN 1193 20 mg/kg i.p. b.i.d. with the first and last of 4 dialysis exchanges with 200 mL Dianeal 1.5% daily. Day 11, before antibiotic treatment commenced, PDE cultures were positive in all rabbits. Day 15, following 4 days of treatment with SYN 1193, no bacterial growth was detected in Rabbits 9 and 10 and 1.3×10^2 CFU/mL were found in the PDE of Rabbit 11. Bacterial strains isolated were identified as *S. aureus* MU7056. Due to catheter related problems, PDE samples were not available for culture from Rabbit 12 days 12-15.

	Rabbit # 9 Colonies/mL	Rabbit # 10 Colonies/mL	Rabbit # 11 Colonies/mL	Rabbit # 12 Colonies/mL
Day 07	No growth	No growth	No growth	No growth
Day 08	No growth	No growth	No growth	6.2×10^5
Day 11	6.0×10^5	1.1×10^5	2×10^4	1×10^6
Day 12	2×10	7.0×10^2	3.4×10^3	No Sample
Day 13	No growth	No growth	2.0×10^3	No Sample
Day 14	No growth	No growth	1.8×10^2	No Sample
Day 15*	No growth	No growth	1.3×10^2	No Sample

Plasma Concentrations and Pharmacokinetic Analysis SYN 1193 in Rabbits 9-12

Plasma concentrations of SYN 1193 for test Rabbits 9 -12 following administration of SYN 1193 20 mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges each day x 4 days are shown in **Figures 4.4 – 4.7 and Table 4.10**. Peritoneal dialysis was carried out four times a day at 0, 4, 8 and 12 hours. Peak concentrations ranged from 8.15 – 12.9 μg/mL with a mean ± SE of 10.33 ± 0.98 μ/mL. The Tmax occurred at 0.42 - 1.0 hr with a mean ± SE of 0.59 ± 0.14 h. The t1/2 ranged from 5.39 – 22.1 hr with a mean ±SD of 11.69 ± 3.6.

Table 4.10 Plasma Concentrations SYN 1193.
Total and free plasma concentrations of SYN 1193 on Day 14, on the fourth day of treatment with 20 mg/kg i.p. b.i.d. with the first dialysis exchange at 0 hr. Two additional exchanges were carried out at 4 hr and 8 hr. SYN 1193 was administered with the 12 hr exchange after plasma and PDE samples were collected.

Rabbit 9			**Rabbit 10**			**Rabbit 11**			**Rabbit 12**		
Time hr	**Total Conc. ug/ml**	**Free Conc μg/mL**	**Time hr**	**Total Conc ug/ml**	**Free Conc μg/mL**	**Actual Time hr**	**Total Conc ug/ml**	**Free Conc μg/mL**	**Time hr**	**Total Conc ug/ml**	**Free Conc ug/ml**
0	0.23	0.05	0	0.41	0.08	0	0.29	0.06	0	0.15	0.3
0.5	8.84	1.77	0.42	10.11	2.02	0.5	12.90	2.58	0.42	10.17	2.03
1	8.15	1.63	1	7.78	1.56	1	11.91	2.38	1	6.45	1.29
2.05	3.43	0.69	2	2.98	0.60	2	4.12	0.82	2	1.92	0.38
4	0.69	0.14	4.08	0.77	0.15	4	0.99	0.20	4	0.43	0.09
8.25	0.41	0.08	8.08	0.72	0.14	8.17	0.39	0.08	8.08	0.24	0.05
12.5	0.35	0.07	12	0.60	0.12	12.25	0.35	0.07	12.25	0.25	0.05
24.17	0.36	0.07	23.5	0.78	0.16	23.25	0.84	0.17	23.58	0.23	0.05
Tmax	1.0 hr		**Tmax**	0.42hr		**Tmax**	0.5hr		**Tmax**	0.42hr	
Cmax	8.15 ug/mL		**Cmax**	10.11 ug/mL		**Cmax**	12.9 ug/mL		**Cmax**	10.17 ug/mL	2.03 μg/mL
t1/2	8.62 hr		**t1/2**	22.1 hr		**t1/2**	5.39 hr		**t1/2**	10.66 hr	

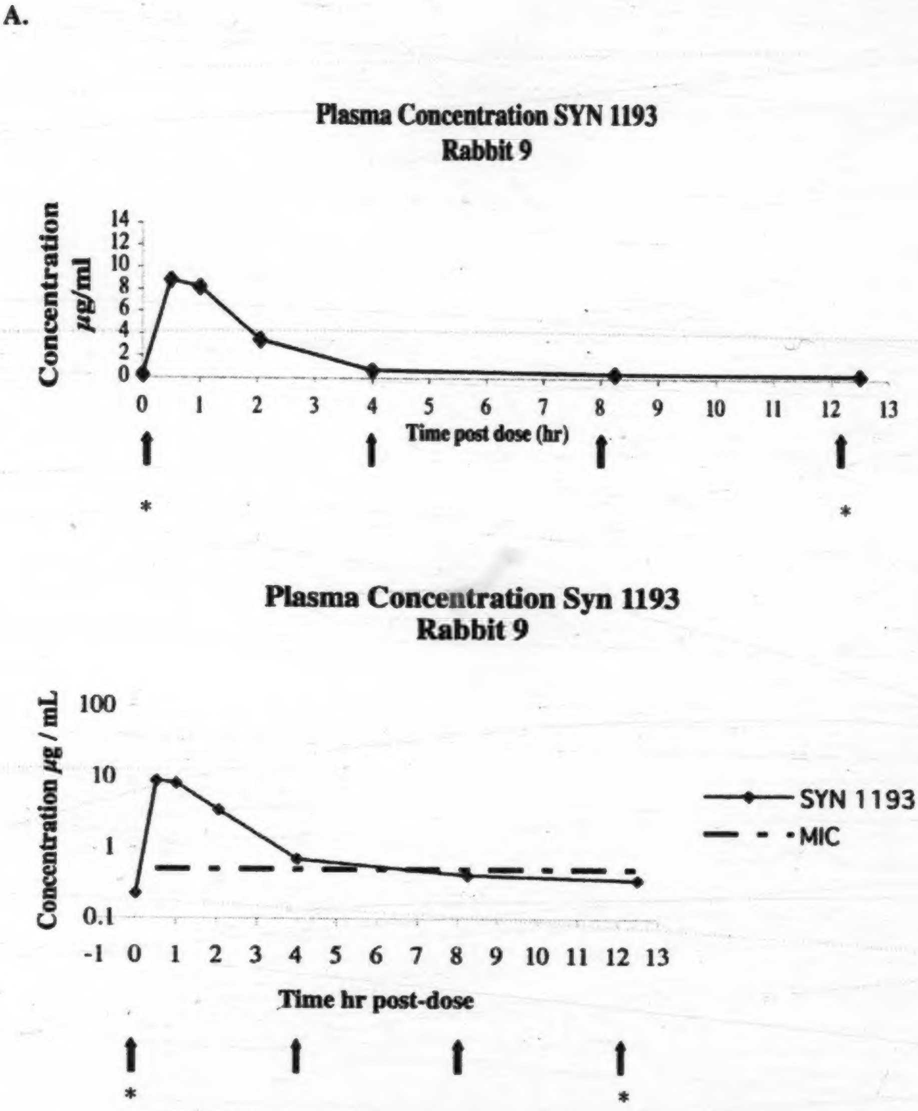


Figure 4.4. **Plasma Concentrations SYN 1193 – Rabbit 9.** Linear (A) and semilogarithmic (B) plots of plasma concentrations of SYN 1193 in Rabbit 9 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 µg/mL) approximately 6 hours of the 12 hour dosing interval. Arrows indicate dialysis exchange times, * indicates time of drug administration.

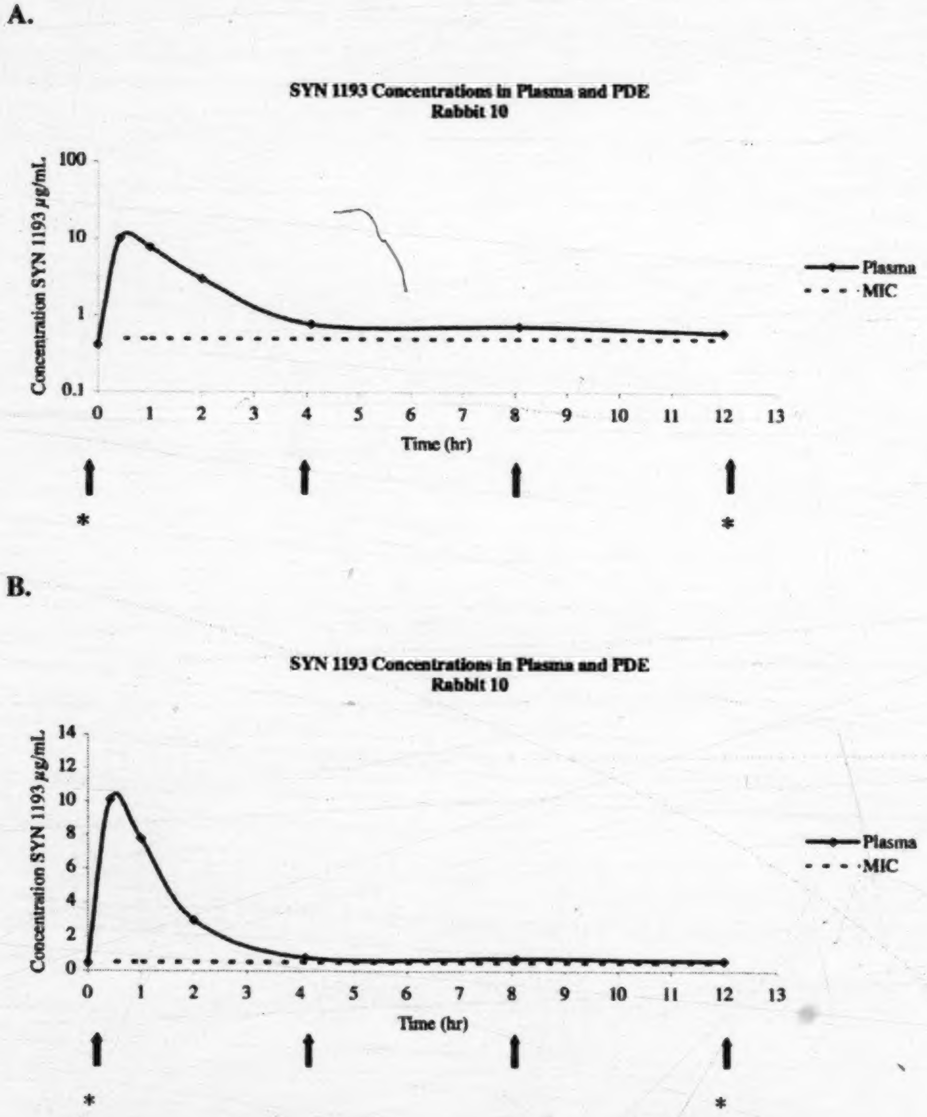


Figure 4.5. **Plasma Concentrations SYN 1993 – Rabbit 10** Linear (A) and semilogarithmic (B) plots of plasma concentrations of SYN 1193 in Rabbit 10 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (0.5 μg/mL) for the entire 12 hr dosing interval. Arrows indicate dialysis times, * indicate drug administration time.

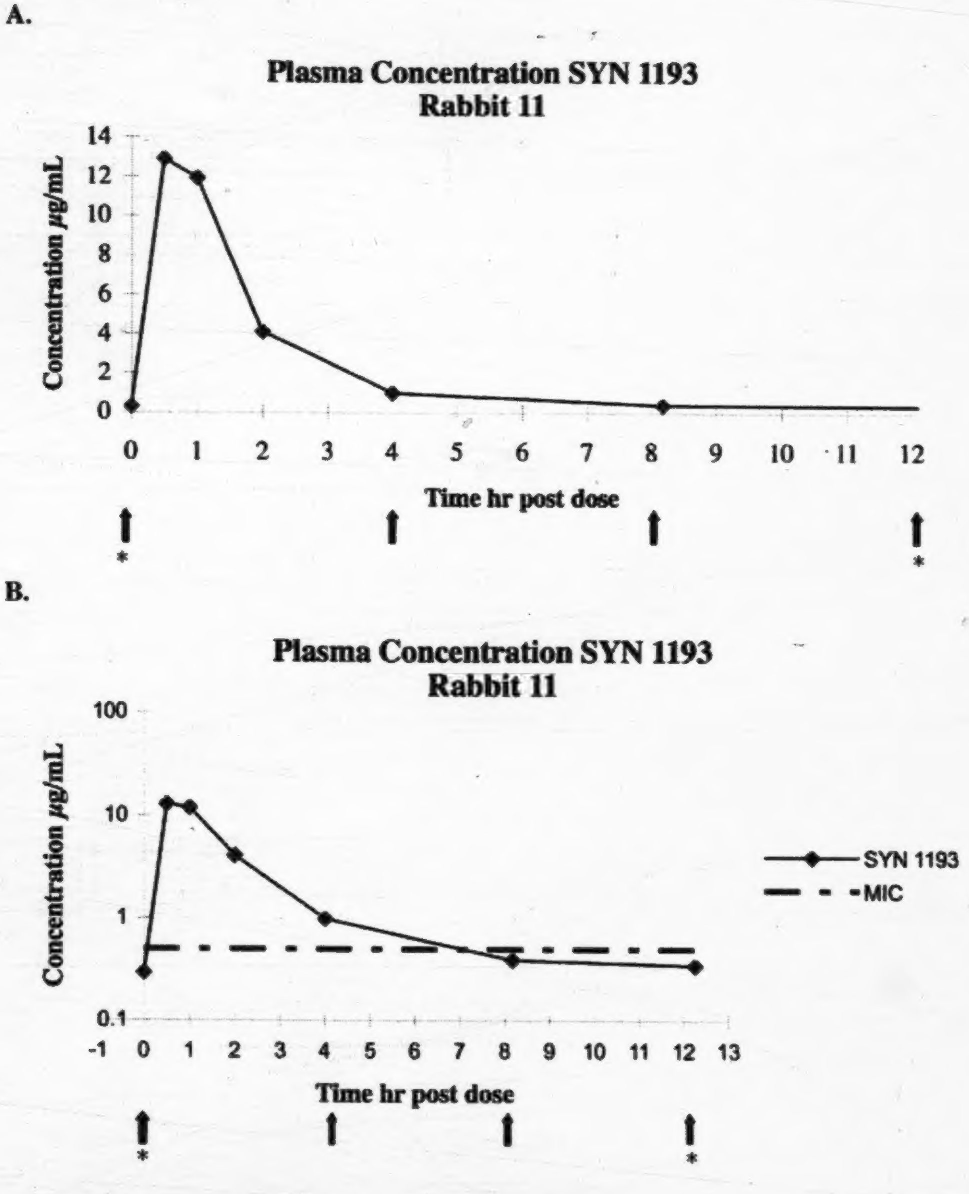


Figure 4.6. Plasma Concentrations SYN 1193 – Rabbit 11
Linear (A) and semilogarithmic (B) plots of plasma concentrations of SYN 1193 in Rabbit 11 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (0.5 µg/mL) for approximately 7 hr of the 12 hour dosing interval. Arrows indicate dialysis times, * indicates SYN 1193 administration times.

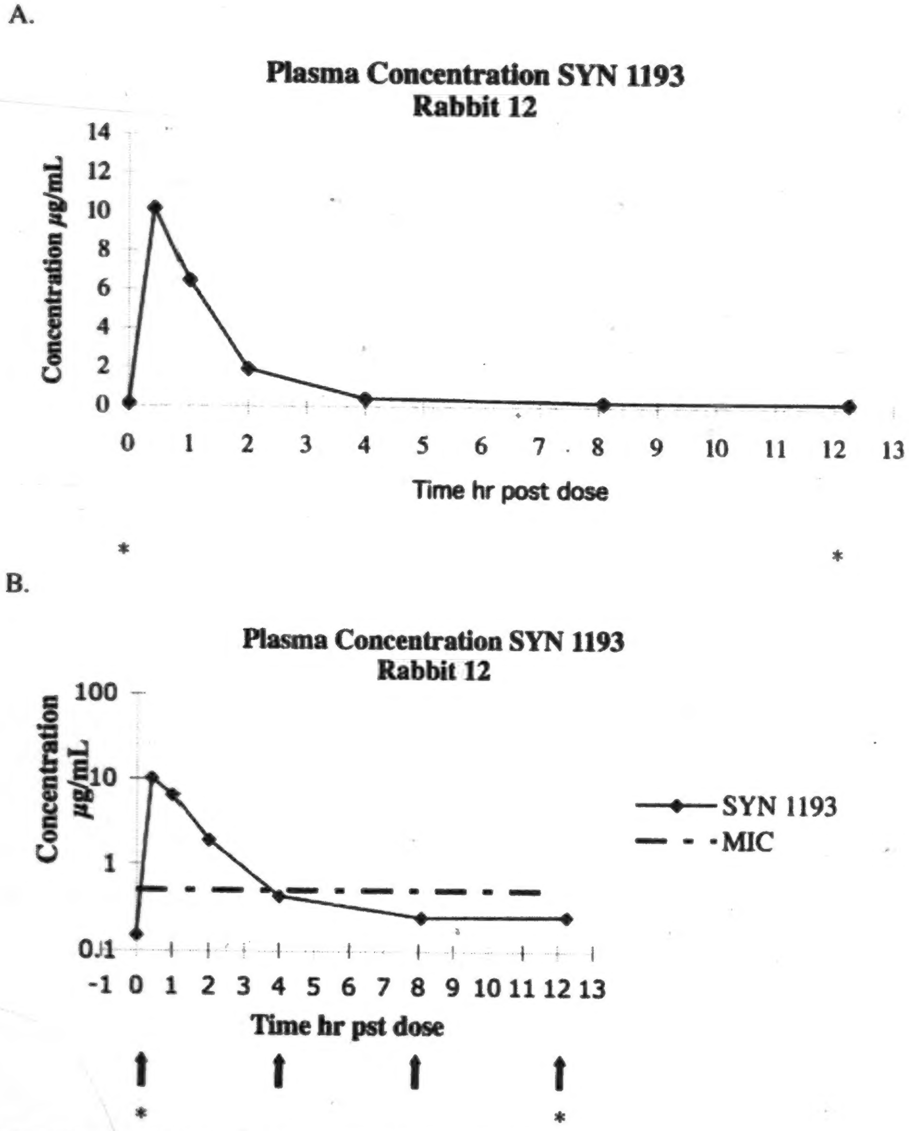


Figure 4.7. **Plasma Concentrations SYN 1193 – Rabbit 12**
Linear (A) and semilogarithmic (B) plots of plasma concentrations of SYN 1193 in Rabbit 12 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (0.5 µg/mL) for only approximately 3.5 hr of the 12 hour dosing interval. Due to a PD catheter malfunction in this rabbit, fluid could only be administered i.p. and not withdrawn. Arrows indicate dialysis exchange times, * indicates SYN 1193 administration times.

PDE Concentrations SYN 1193

Concentrations of SYN 1193 for Rabbits 9 – 11 following 4 days of treatment with SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr with dialysis exchanges of 200 mL Dianeal 1.5% at 0, 4, 8 and 12 hr are shown in **Table 4.11** and **Figures 4.8 – 4.10**. PDE concentrations were not available for Rabbit 12 due to a blockage of the PD catheter that allowed fluid with drug to be introduced into the peritoneal cavity but not withdrawn.

Maximum concentrations ranging from 89.8 – 115.7 μg/mL with a mean ± SD of 104.03 ± 7.59 were measured at 0.5 – 1 hr. Tmax ranged from 0.5 – 1 hr with a mean ± SE of 0.83 ±0.17.

Concentrations of SYN 1193 were maintained above the MIC of SYN 1193 for *S. aureus* MU7056 of 0.5 μg/mL for 9.5 hr in Rabbit 10 and 10 hr in Rabbits 10 and 11. It is interesting to note that in Rabbits 10 and 11, concentrations of SYN 1193 in PDE fell to concentrations of 0.39 and 0.46 μg/mL below the MIC of 0.5 μg/mL with the 8 hr dialysis exchange, but gradually increased to concentrations above the MIC of 0.5 μg/mL by 10 – 10.5 hr and continued to increase to concentrations of 0.62 and 0.79 μg/mL by the end of the 12 hour dosing interval.

Table 4.11 PDE Concentrations SYN 1193 Rabbits 9 - 11

Rabbit 9 **PDE Thursday**		**Rabbit 10** **PDE Thursday**		**Rabbit 11** **PDE Thursday**	
Time Post Dose (hr)	PDE Concentration SYN 1193 μg/ml	Time Post Dose (hr)	PDE Concentration SYN 1193 μg/ml	Time Post Dose (hr)	PDE Concentration SYN 1193 μg/ml
-0.83		-.1		0	0.8
0.583	14.3	0.5	88.8	0.5	106.6
1	115.7	1	89.8	1	88.6
2.03	82.2	2	23.8	2.03	24
3.83	3.2	3.83	1.2	3.93	5.2
4.08	1.038	4.08	1.8	3.98	4.7
8.08	0.899	7.83	0.519	8.08	0.82
8.33	0.622	8.08	0.29	8.25	0.357
12.42	0.379	11.92	0.62	12.33	0.794
12.62	113.406	12.25	129.4	12.83	138.75
24	4.39		no sample	24	5.98
Tmax	**1 hr**	**Tmax**	**1 hr**	**Tmax**	**0.5 hr**
Cmax	**115.7 μg/ml**	**Cmax**	**89.8 μg/ ml**	**Cmax**	**106.6μg/ml**

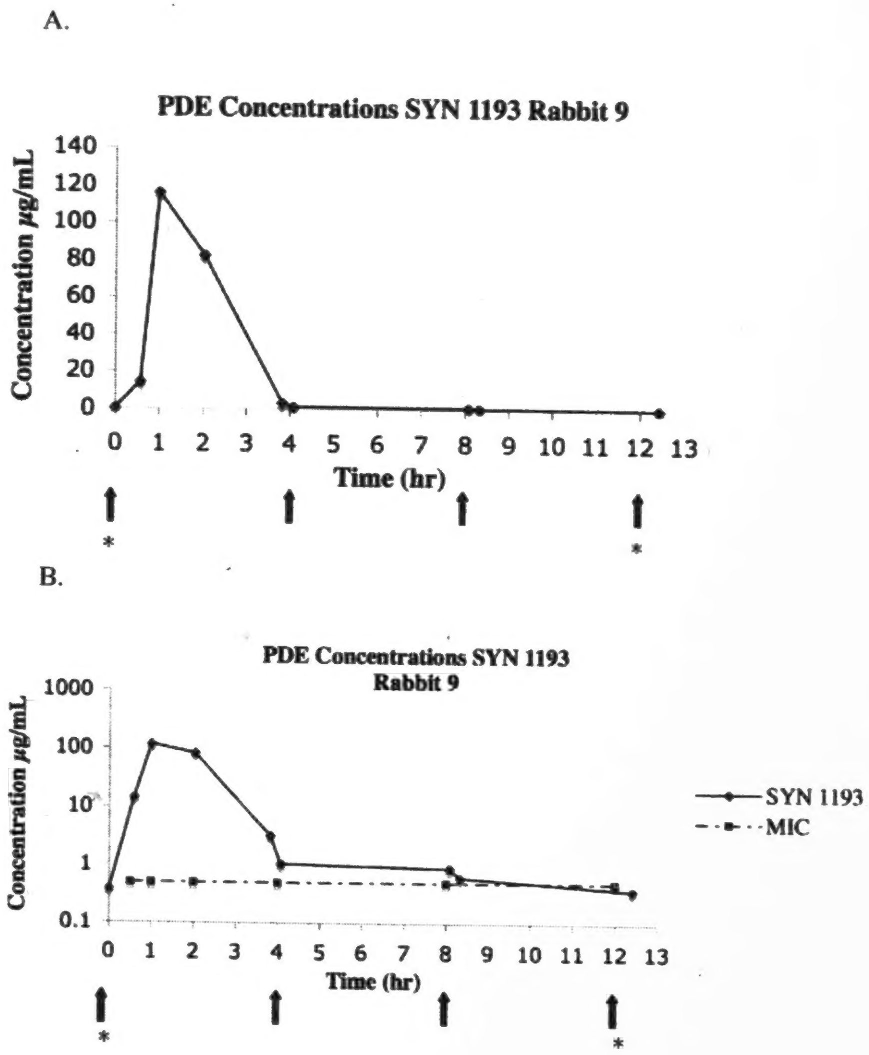


***Figure 4.8.* PDE Concentrations SYN 1193 Rabbit 9.**
Linear (A) and Log-linear (B) plots of PDE concentrations of SYN 1193 in Rabbit 9 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (0.5 µg/mL) for 10 hr of the 12 hour dosing interval. Arrows indicates times of dialysis exchanges, * indicates administration times of SYN 1193.

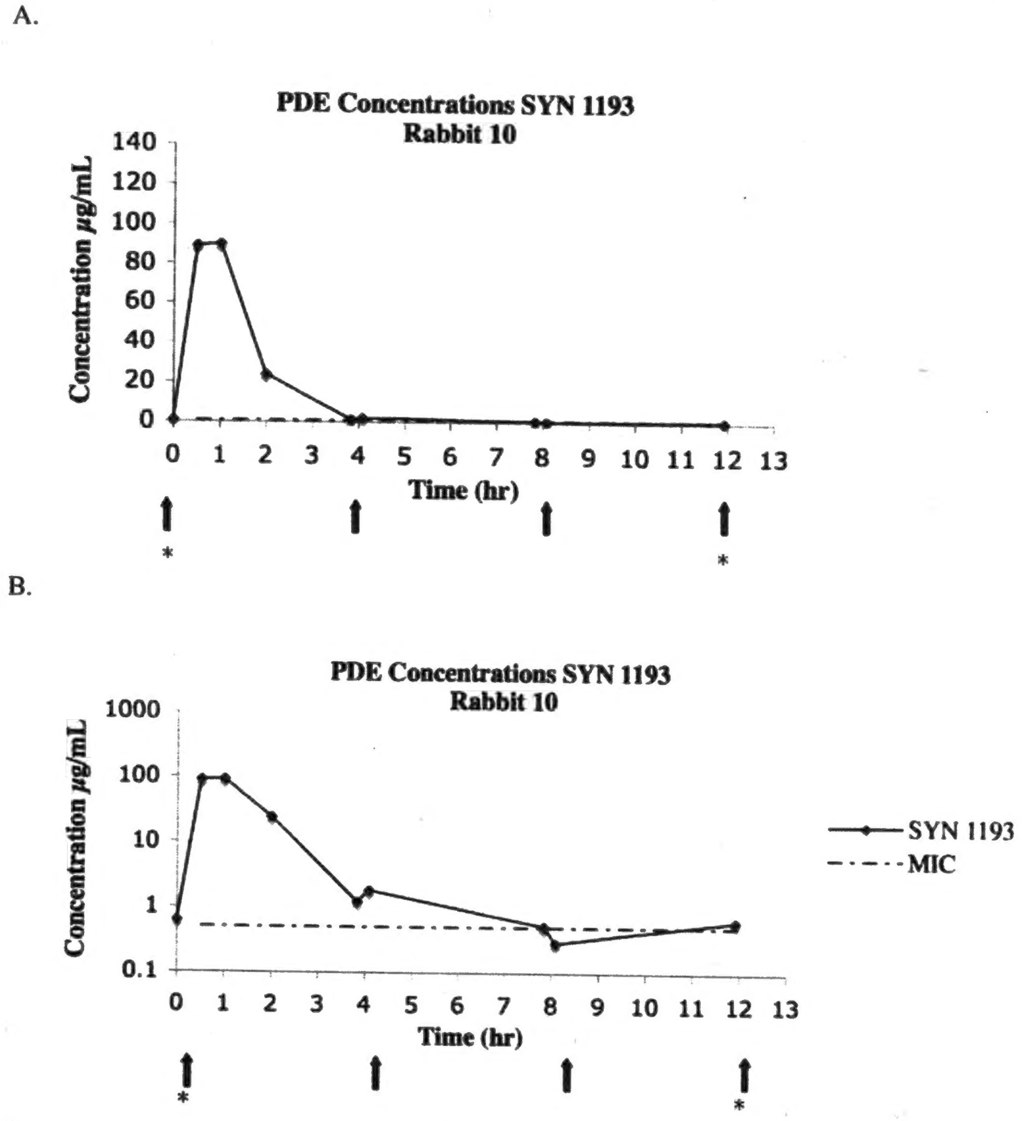


***Figure 4.9*. PDE Concentrations SYN 1193 Rabbit 10**
Linear (A) and Log-linear (B) plots of PDE concentrations of SYN 1193 in Rabbit 10 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (0.5 μg/mL) most of the 12 hour dosing interval. It is interesting to note that although PDE concentrations dropped below the MIC following dialysis at 8 hr, PDE concentrations once again exceeded the MIC by 10.5 hr. Arrows indicate times of dialysis exchanges, * indicates times of SYN 1193 administration.

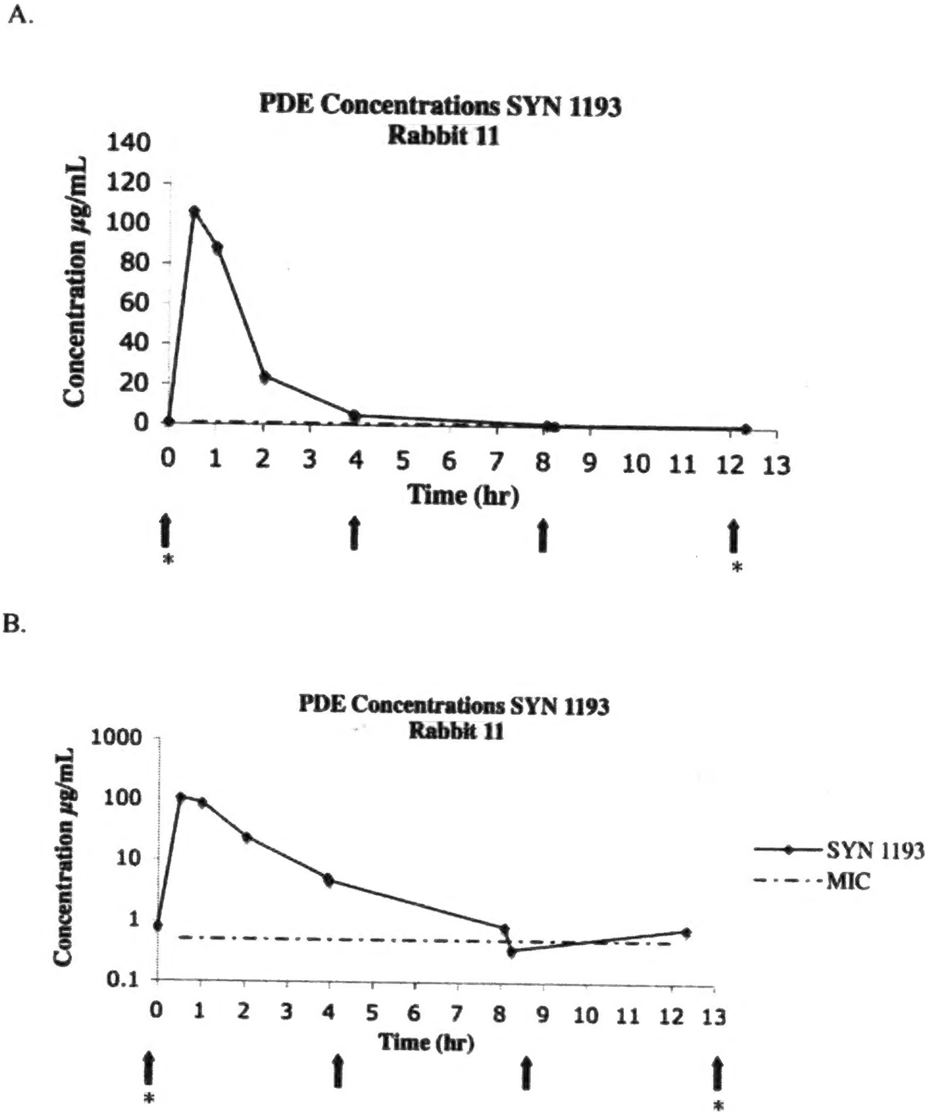


Figure 4.10 **. PDE Concentrations SYN 1193 Rabbit 11.** Linear (A) and Log-linear (B) plots of PDE concentrations of SYN 1193 in Rabbit 11 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (0.5 μg/mL) for 10 hr of the 12 hour dosing interval. As in Rabbit 10, although PDE concentrations drop below the MIC with the 8 hr dialysis, they rise above the MIC by 10 hr and remain above the MIC until the end of the 12 hr dosing interval.

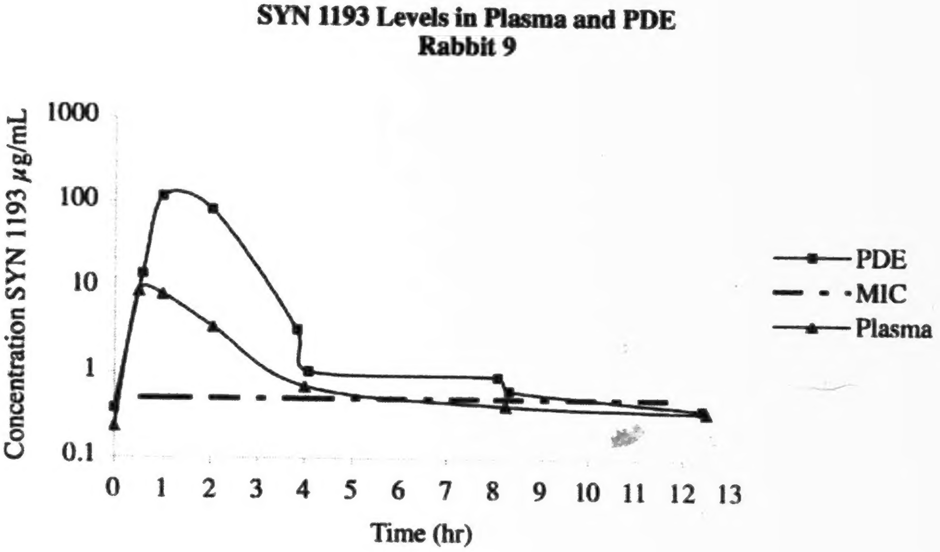


Figure 4.11. **Plasma and PDE Concentrations SYN 1193 Rabbit 9** Semilogarithmic plots of plasma and PDE concentrations of SYN 1193 in Rabbit 9 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days relative to the MIC of SYN 1193 for *S. aureus* MU7056. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. High initial concentrations of SYN 1193 in PDE provided plasma concentrations which remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 μg/mL) for approximately 6 hr of the 12 hour dosing interval. PDE concentrations remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 μg/mL) for approximately 10 hr.

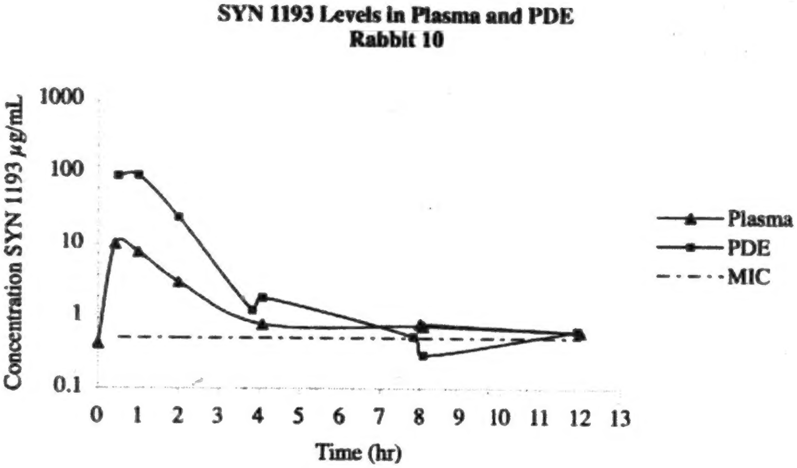


Figure 4.12 **Plasma and PDE Concentrations SYN 1193 Rabbit 10**
Semilogarithmic (B) plots depict plasma and PDE concentrations of SYN 1193 relative to the MIC of SYN 1193 for *S. aureus* MU7056 in Rabbit 10 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. High initial concentrations of SYN 1193 in PDE provided plasma concentrations which remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 μg/mL) for the entire 12 hr dosing interval. PDE concentrations remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 μg/mL) for approximately 8 hr but dropped to 0.29 following the 8 hr dialysis exchange and rose to a level of 0.62 above the MIC by the end of the dosing interval.

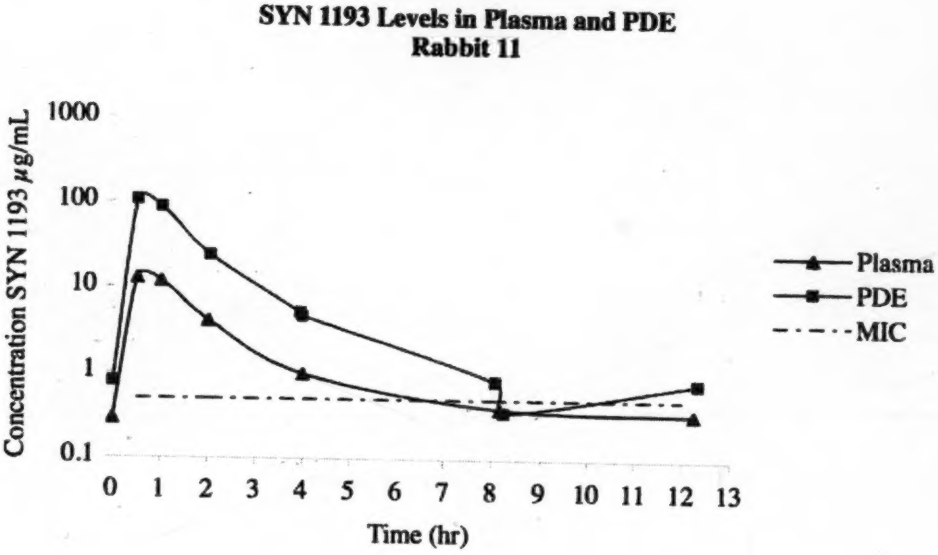


Figure 4.13. **Plasma and PDE Concentrations SYN 1193 Rabbit 11** Semilogarithmic plots show plasma and PDE concentrations of SYN 1193 in Rabbit 11 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis was carried out at 0, 4, 8 and 12 hours each day. High initial concentrations of SYN 1193 in PDE provided plasma concentrations which remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 μg/mL) for approximately 7 hrs of the 12 hr dosing interval. PDE concentrations remained above the MIC of SYN 1193 for *S. aureus* MU 7056 (0.5 μg/mL) for approximately 8 hr and rose above the MIC by 10 hr to a level of 0.79 above the MIC by the end of the dosing interval.

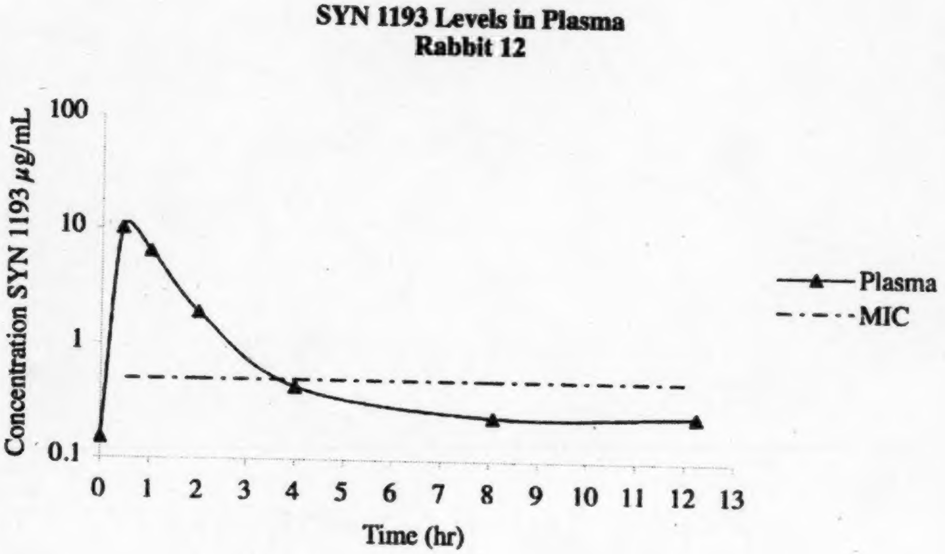


Figure 4.14 **Plasma Concentrations SYN 1193 Rabbit 12.**
A semilogarithmic plot shows plasma concentrations of SYN 1193 in Rabbit 12 following administration of SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days relative to the MIC. Due to a PD catheter malfunction, fluid could only be administered i.p. and not withdrawn. As a result the rabbit received the dose of SYN 1193 i.p. b.i.d. at 08:00 and 20:00, but no further exchanges were carried out. Despite this, plasma concentrations only remained above the MIC of *S. aureus* MU 7056 (0.5 μg/mL) for approximately 3.5 hr of the 12 hr dosing interval.

Correlation Between SYN 1193 Concentrations in Plasma with PDE

Regression analysis showed significant $p < 0.0001$ and excellent ($R^2 = 0.74$) correlations between plasma and PDE concentrations in Rabbits 9 -12 indicating that at any time the concentration in the plasma fluid reflects the concentration in the PDE A linear regression plots is shown in **Figure 4.15**.

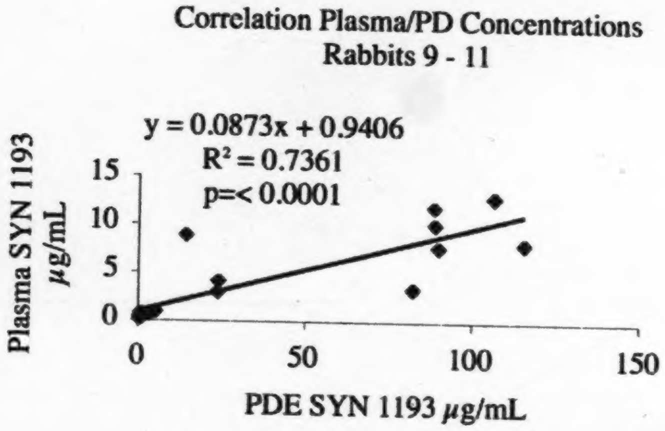


Figure 4.15. **Correlation Between PDE and Plasma Concentrations SYN 1193.** An excellent correlation was found between PDE and Plasma Concentrations SYN 1193 $R2 = 0.731$, $p < 0.0001$.

Urinary Excretion SYN 1193

The 24 h urinary excretion of SYN 1193 during the days 14-15 period was minimal ranging from 0.42 – 7.19% of the total daily dose as reported in **Table 4.12**. This is consistent with preliminary studies done by Synphar in rats finding 1% excreted in urine and > 90% excreted in bile.

Table 4.12. **Urinary Excretion of SYN 1193.** Twenty-four hour urinary excretion of SYN 1193 ranged from 0.42 – 7.19% of the total 24 hour dose. This is consistent with preliminary studies done by Synphar in rat which showed 1% excreted in urine and > 90% excreted in bile.

	Sample	**Time**	**Calculated Concentration µg/mL**	**Volume mL**	**Amount µg**	**24 hr Dose mg**	**% 24hr Dose in Urine**
Rabbit 9							
	1-12 hr	7:45-20:55	22.43	250	5609.33		
	12-24 hr	20:55-07:30	14.26	150	2139.09		
				24 hr Total	**7748.42**	135.2	**5.73**
Rabbit 10							
	1-12 hr	8:15-20:57	10.79	200	2157.06		
	12-24 hr	20:55-07:30	21.61	350	7564.91		
				24 hr Total	**9721.97**	128	**7.19**
Rabbit 11							
	1-12 hr	8:15-20:57	17.53	125	2191.55		
	12-24 hr	20:57-07:30	16.96	175	2968.8		
				24 hr Total	**5160.35**	136	**3.82**
Rabbit 12							
	1-12 hr	8:25-21:00	11.31	50	565.76		
	12-24 hr	21:00-07:30	0.47	2	0		
				24 hr Total	**565.76**	136.8	**0.42**

Peak/MIC and AUC$_{24}$/ MIC Ratios in Plasma for Rabbits Receiving SYN 1193

Peak plasma concentrations ranged from 8.15 – 12.9 μg/mL with a mean ± SE of 10.33 ± 0.98 μg/mL. The MIC of SYN 1193 for the infecting strain *S. aureus* MU7056 is 0.5 μg/mL. Resultant peak/MIC ratios were 16.3 - 25.8 with a mean ± SE of 20.67 ± 1.95.

The 24 hr AUC of SYN 1193 in plasma ranged from 34.42 to 70.40 μg/mL*hr with a mean ± SE of 48.39 ± 7.79 μg/mL*hr. Resultant AUC$_{24}$/MIC ratios ranged from 68.83 – 140.80 with a mean ± SE of 96.57 ± 15.67.

Peak/MIC and AUC$_{24}$/MIC ratios in plasma for rabbits receiving SYN 1193 are shown in **Table 4.13**.

Peak/MIC and AUC$_{24}$/MIC Ratios in PDE for Rabbits Receiving SYN 1193

Peak concentrations in PDE ranged from 89.8 – 115.7 μg/mL with a mean ± SE of 104.03 ± 7.59 μg/mL. The MIC of SYN 1193 for the infecting strain *S. aureus* MU7056 is 0.5 μg/mL. Resultant Peak/MIC ratios in PDE of 179.6 – 231.4 with a mean ± SE of 208.06 ± 15.17.

The 24 hr AUC of SYN 1193 in PDE ranged from 153.24 to 216.98 μg/mL*hr with a mean ± SE of 181.87 ± 16.68 μg/mL*hr. Resultant AUC$_{24}$/MIC ratios ranged from 306.48 to 433.96 with a mean ± SE of 363.74 ± 37.37.

Peak/MIC and AUC$_{24}$/MIC ratios in PDE for rabbits receiving SYN 1193 20mg/kg i.p. b.i.d. with the first and last of 4 exchanges each day for 4 days are shown in **Table 4.14**.

Table 4.13. Peak/MIC and AUC_{24}/MIC Ratios in Plasma for Rabbits Receiving SYN 1193 x 4 days.

	t_{max} (hr)	C_{max} SYN 1193 Plasma (μg/mL)	Peak/MIC SYN 1193 Plasma	24 hr AUC SYN 1193 Plasma (μg/mL*hr^{-1})	24 hr AUC_{24}/MIC SYN 1193 Plasma	T>MIC Plasma (hr)
Rabbit 9	1	8.15	16.3	41.45	82.90	6
Rabbit 10	0.42	10.11	20.22	47.3	94.27	12
Rabbit 11	0.5	12.9	25.8	70.40	140.80	7
Rabbit 12	0.42	10.17	20.34	34.42	68.83	3.5
Mean ± SE	0.59 ± 0.14	10.33 ± 0.98	20.67 ± 1.95	48.39 ± 7.79	96.57 ± 15.67	7.13 ± 1.78

Table 4.14. Peak/MIC and AUC_{24}/MIC Ratios in PDE for Rabbits Receiving SYN 1193.

	C_{max} (μg/mL) SYN 1193 PDE	Peak/MIC SYN 1193 PDE	24 hr AUC (μg/mL*hr) SYN 1193 PDE	AUC_{24}/MIC SYN 1193 PDE	T>MIC (hr)
Rabbit 9	115.7	231.4	216.98	433.96	10
Rabbit 10	89.8	179.6	153.24	306.48	9
Rabbit 11	106.6	213.2	175.39	350.78	8
Rabbit 12	PD Catheter Plugged to Withdrawal - PDE Samples Not Available				
Mean ± SE	104.03 ± 7.59	208.06 ± 15.17	181.87 ± 18.68	363.74 ± 37.37	9 ± 0.58

Tissue and PD Catheter Sample Culture and S.E.M. Results
Rabbits 9-12 Treated with SYN 1193

Tissue and PD catheter sample culture results following treatment with SYN 1193 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days with peritoneal dialysis exchanges at 0, 4, 8 and 12 hours each day are shown in **Table 4.15 and Table 4.16**.

No growth was detected at any site in Rabbit 9, however S.E.M. of the external catheter was positive for cocci indicative of the presence of adherent bacteria on the PD catheter.

Rabbits 9-12 showed no growth at a number of sites. Bacterial growth with *S. aureus* MU7056 was the only pathogen detected at all sites with the exception of the peritoneal wall tissue which showed growth of diptheroids and coagulase negative staphylococci. Bacterial growth ranged from 6.0×10^1 to 4.6×10^5 CFU/mL/g tissue.

S.E.M. was negative for the external catheter of Rabbit 9 and the internal catheter of Rabbit 10, but positive for internal and external catheter samples for Rabbits 9 and 10 respectively.

S.E.M. was negative for the external catheter sample in Rabbit 10 that showed positive growth of *S. aureus* MU7056 on culture of an adjacent sample of external catheter.

S.E.M. was positive for the external catheter in Rabbit 9, and internal catheter samples of Rabbits 10 and 12 where no growth was detected on culture indicating the presence of adherent bacteria.

Table 4.15. **Tissue/Catheter Sample Culture Results Post Treatment With SYN 1193.** Colony Counts *S. aureus* MU7056 (CFU / mL /g tissue) are shown along with bacterial taxonomic identification post-treatment with SYN 1193 20 mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges with 250 ml Dianeal 1.5%. Coagulase negative staphylococci (CNS) and diptheroids were identified in addition to *S. aureus* MU7056.

	Rabbit # 9	**Rabbit # 10**	**Rabbit # 11**	**Rabbit # 12**
Dacron Cuff	No growth	4×10^5	4.6×10^5	6.0×10^1
Internal Catheter	No growth	No growth	2.3×10^2	No growth
External Catheter	No growth	1.1×10^2	6.0×10^1	4.9×10^2
Peritoneal Wall Tissue	No growth	5.9×10^3 CNS, Diptheroids	5.6×10^1	No growth
Peritoneal Wall Tissue	No growth	No growth	1.5×10^2	No growth
Subcutaneous Tunnel 10 cm	No growth	No growth	No growth	6.6×10^2
Subcutaneous Tunnel 20 cm	No growth	No growth	No growth	4.5×10^1

Abbreviations: CNS = coagulase negative staphylococci

Table 4.16. Scanning Electron Microscopy and bacterial growth (CFU/g/mL) of Internal and External Catheter Samples in Rabbits Treated with SYN 1193.

	Rabbit 9	**Rabbit 10**	**Rabbit11**	**Rabbit 12**
S.E.M. Internal Catheter (Peritoneal Cavity)	Negative	Cocci +/-	Cocci +/-	Cocci +/-
Culture (CFU/mL) Internal Catheter (Peritoneal Cavity)	No growth	No growth	2.3×10^{2} S. A. MU7056	No growth
S.E.M. External Catheter (Tunnel)	Cocci	Negative	Cocci +/-	Cocci +/-
Culture (CFU/mL) External Catheter (CFU/mL) (Tunnel)	No growth	1.1×10^{2} S. A. MU7056	6.0×10^{1} S. A. MU7056	4.9×10^{2} S. A. MU7056

Log_{10} Reduction CFU/mL in PDE in SYN 1193 Treated Rabbits

Day 11, following inoculation and 2 dialysis treatments Day 10 to ensure development of peritonitis and before initiation of treatment with SYN1193, $\log_{10}$ bacterial CFU/mL in PDE in Rabbits 9 - 11 ranged from 2.19 – 5.78 with a mean of 5.04. Due to a malfunctioning catheter in Rabbit 12 that would not allow withdrawal of fluids, samples were not available to determine overall $\log_{10}$ reduction in bacterial growth.

Day 15, following 4 days of treatment with SYN 1193 20 mg/kg b.i.d. i.p. in the first and last of 4 dialysis exchanges per day with 200 ml Dianeal 1.5%, $\log_{10}$ CFU/mL were reduced to 0 in Rabbits 9 and 10 and 2.11 in Rabbit 11 for an overall mean of 0.7 $\log_{10}$ CFU/mL.

The $\log_{10}$ reduction in CFU/mL over the treatment period ranged from 2.19 – 5.78 with a mean ± SE of 4.34 ± 1.09. A 4 $\log_{10}$ reduction is indicative of a 99.99% reduction in bacterial growth.

Initial bacterial growth in PDE 9-12 days 11 and 15 are shown as $\log_{10}$ CFU/mL and $\log_{10}$ reductions over the 4 day treatment period are reported for Rabbits 9-12 in **Table 4.17**.

Time to Reduction of Bacterial Growth by One Log_{10} With SYN 1193

Reduction in bacteria growth by one $\log_{10}$ was achieved in <1day in Rabbits 9 and 10 and in ≤2days in Rabbit 11. Due to a malfunctioning catheter, samples were not available to determine time to reduction of bacterial growth by 1 $\log_{10}$ in Rabbit 12.

Table 4.17. Log_{10} Reduction CFU/mL in PDE of SYN 1193 Treated Rabbits

Initial bacterial growth in PDE of Rabbits 9-12 Days 11 and 15 are shown as $\log_{10}$ CFU/mL and $\log_{10}$ reductions and the mean $\log_{10}$ reduction are shown over the 4 day treatment period.

	Rabbit 9	**Rabbit 10**	**Rabbit 11**	**Rabbit 12**	**Mean**
	Log_{10} CFU/mL	Log_{10} CFU/mL	Log_{10} CFU/mL	Log_{10} CFU/mL	Log_{10} CFU/mL
Day 11	5.78	5.04	4.30	(6.0)	5.04
Day 15	0	0	2.11	Sample not available	0.70
Log_{10} Reduction	**5.78**	**5.04**	**2.19**		**4.34 ± 1.09** (Mean ± SE)

D/P Ratio SYN 1193

The D/P ratio (the ratio of concentration of the drug in the dialysate to the concentration of drug in plasma at a specified time after i.p. administration) is indicative of the permeability of the membrane and the ability of the drug to cross the membrane. The D/P ratios of SYN 1193 following administration of SYN 1193 20 mg/kg i.p. q12h with the first and last of 4 dialysis exchanges with 250 ml Dianeal 1.5% are depicted in **Figure 4.16** and **Table 4.18.** Variability in membrane permeability may explain some of the variability in pharmacokinetic parameters.

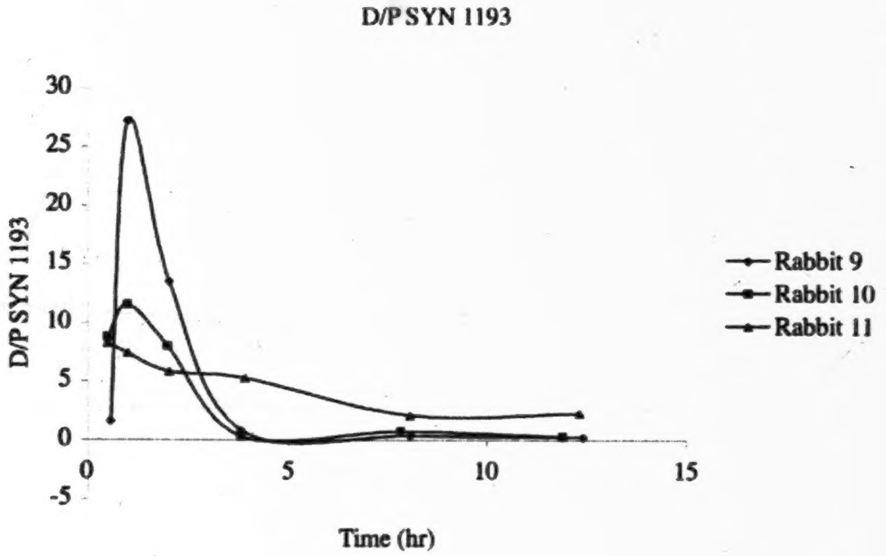


Figure 4.16. Comparison D/P Ratios SYN 1193 Rabbits 9, 10 and 11
A graph of D/P ratios for SYN 1193 over time for Rabbits 9 10 and 11 shows the variability in the permeability of the peritoneal membrane in these rabbits.

Table 4.18. D/P Ratios SYN 1193 – Rabbits 9 -11

Rabbit 9 Time Post-Dose hr	PD Conc. μg/mL	Plasma Conc. μg/mL	D/P Ratio	Rabbit 10 Time Post-Dose hr	PD Conc. μg/mL	Plasma Conc. μg/mL	D/P Ratio	Rabbit 11 Time Post-Dose hr	PD Conc. μg/mL	Plasma Conc. μg/mL	D/P Ratio
-0.83				-0.1		0.41		0	0.80	0.29	
0.583	14.3	8.84	1.62	0.5	88.8	10.11	8.78	0.5	106.60	12.9	8.26
1	115.7	4.25	27.22	1	89.8	7.78	11.54	1	88.60	11.91	7.44
2.03	82.2	6.08	13.52	2	23.8	2.98	7.99	2.03	24.00	4.12	5.83
3.83	3.2	4.02	0.80	3.83	1.2	3.9	0.31	3.93	5.20	0.99	5.25
4.08	1			4.08	1.8			3.98	4.70		
8.08	0.9	2.35	0.38	7.83	0.52	0.72	0.72	8.08	0.82	0.39	2.10
8.33	0.62			8.08	0.29			8.25	0.36		
12.42	0.38	1.62	0.23	11.92	0.69	2.59	0.27	12.33	0.79	0.35	2.27
12.62	113.41			12.25	129.4			12.83	138.75		
24	4.39			no sample				24	5.98		

Results Rabbits 13A-16A Treated with Vancomycin

Rabbits 13 – 16 Inoculation

Rabbits 13 –16 were inoculated with *S. aureus* MU7056 days 6 and 7 and dialysed twice on day 10 to ensure development of peritonitis in preparation for treatment with vancomycin 20 mg/kg i.p. b.i.d..

Day 8, it was noticed that Rabbit 16 had a large hole in the catheter, just below the level of the skin at the exit site. This was likely caused at the time of surgery when the purse string sutures were put in to secure the catheter. An attempt was made to repair the catheter. The rabbit was anaesthetized with ketamine cocktail. The existing catheter was cut proximal to the hole and an extension added to the existing catheter. Although this had disrupted the healing process at the catheter exit site, the exit site was inoculated and the rabbit dialysed on Day 10 as per protocol. Day 11, Rabbit 16 was found dead in its cage.

Day 10, when the two dialysis exchanges were attempted to induce peritonitis, it was noted in all rabbits that although fluid could be introduced into the peritoneal cavity, it could not be withdrawn. Day 11, Vancomycin treatment 20 mg/kg i.p. b.i.d. was initiated, but the rabbits were only dialysed twice a day due to abdominal distention in all rabbits. It was hoped that with treatment, the catheters would begin to work again. Day 12, the rabbits seemed much improved but since the catheters still would not withdraw, the rabbits were sacrificed the usual necropsy procedures performed. At the time of necropsy, it was apparent that pus and fibrinous tissue surrounding the catheter tip and adhering to the intestines had blocked the catheter tips.

These experiments were repeated with 4 rabbits 13A to 16A as per protocol.

Rabbits 13A – 16A Receiving Vancomycin

PD catheters were implanted in Rabbits 13A-16A Day 1 as previously described.

Day 3, Rabbit 15A was initially not eating or drinking and had minimal urine output, but improved later. By Day 4, Rabbit 16A had chewed the end of its PD catheter sometime during the weekend leaving the catheter open to contamination. The catheter was repaired with the addition of an extension.

Rabbits 13A - 16A were inoculated with *S. aureus* MU7056 days 6 and 7 and dialysed twice on day 10 at 08:00 and 20:00 hr with 200 mL Dianeal 1.5% to ensure development of peritonitis. Day 10, treatment with vancomycin 20 mg/kg i.p. b.i.d. was initiated and peritoneal dialysis continued q.i.d. as previously described.

Day 11, fluid could not be withdrawn from the PD catheter of Rabbit 16A possibly due to the previous manipulation of the PD catheter or due to the response to the infectious process in the peritoneal cavity. To avoid overfilling the peritoneal cavity, 200mL Dianeal 1.5% was administered with the vancomycin dose and 0:800 and 20:00 only with no withdrawal of fluid at any time. Day 14, Rabbit 16A pulled out his PD catheter.

Peritoneal Effluent Cultures

Rabbits 13A – 16A Pre- and Post-Treatment with Vancomycin

Bacterial growth was detected Day 11, prior to initiation of vancomycin treatment in the PDE of Rabbits 13A – 15A with colony counts ranging from 6×10^{2} to 1.4 x

10^5 CFU / mL **(Table 4.19)**. No sample was attainable for Rabbit 16A due to a malfunctioning PD catheter.

By Day 13, following 2 days of vancomycin treatment, no growth was detected the PDE of any of the rabbits and the PDE remained clear of bacterial growth for the remainder of the study.

Table 4.20. ***PDE Cultures Pre and Post Treatment with Vancomycin***

Rabbits 13A – 15A had positive PDE cultures by Day 10 when administration of vancomycin 20 mg/kg q12h i.p. q12h in the first and last of 4 dialysis exchanges daily was initiated. By Day 12, following 2 days of vancomycin treatment, PDE cultures were negative and remained negative for the rest of the experiment. Due to catheter problems with Rabbit 16A PDE samples were not available for culture.

	Rabbit # 13A	**Rabbit # 14A**	**Rabbit # 15A**	**Rabbit # 16A**
Day 06	No growth	No growth	No growth	No growth
Day 07	No growth	No growth	No growth	No growth
Day 11	9×10^3	6×10^2	1.4×10^5	No growth
Day 12	2.7×10^2	9.0×10^1	3.0×10^2	No sample
Day 13	No growth	No growth	No growth	No sample
Day 14	No growth	No growth	No growth	No sample
Day 15*	No growth	No growth	No growth	No sample

* Necropsies performed Day 15 on all rabbits.

Plasma Concentrations Vancomycin in Rabbits 13A – 16A

Plasma concentrations of vancomycin for test Rabbits 13A – 16A following administration of vancomycin 20 mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges each day x 4 days are shown in **Table 4.20** and **Figures 4.17 – 4.20**. Peritoneal dialysis was carried out four times a day at 0, 4, 8 and 12 hours.

Peak plasma concentrations of vancomycin ranged from 19.5 – 24.5 μg/mL with a mean ± SE of 22.8 ± 1.65 μ/mL in Rabbits 13A – 15A. Rabbit 16A received vancomycin 20 mg/kg i.p at 0:800 and 20:00 hr but, due to catheter malfunction which would allow fluid and drug to be administered but not withdrawn, did not have any withdrawal of fluid. The resulting peak plasma concentration of vancomycin in Rabbit 16A was 33.7 μg/mL.

T_{max} occurred at 1.0 – 2.0 hr with a mean ± SE of 1.5 ± 0.29 h.

Plasma Concentrations of Vancomycin Rabbits 13A – 16

Table 4.20. Plasma vancomycin concentrations relative to the time of drug administration following administration of Vancomycin 20 mg/kg i.p. b.i.d. x 4. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory.

Rabbit 13A		**Rabbit 14A**		**Rabbit 15A**		**Rabbit 16A**	
Time hr	Plasma Concentration μg/ml	Time hr	Plasma Concentration μg/ml	Time hr	Plasma Concentration μg/ml	Time hr	Plasma Concentration μg/ml
0.08	7.7	-0.13	3.8	-0.08	<3	0	3
0.16	22.2	0.45	14	0.42	18.4	0.47	30
1	24.5	0.87	18.9	0.92	23	1	33.7
2	24.4	2	19.5	2	24.4	2	31.4
3.83	17.8	4	16.8	4	16.9	4	17.3
8.08	3.5	8	<3	7.83	3.8	7.8	3.3
11.83	<3.0	12.3	<3	11.45	<3	11.7	<3
23.33	<3.0	11.5	<3	11.08	3.8		
Tmax	1.0 hr	**Tmax**	2 hr	**Tmax**	2 hr	**Tmax**	1 hr
Cmax	24.5 μg/ml	**Cmax**	19.5 μg/ml	**Cmax**	24.4 μg/ml	**Cmax**	33.7 μg/ml

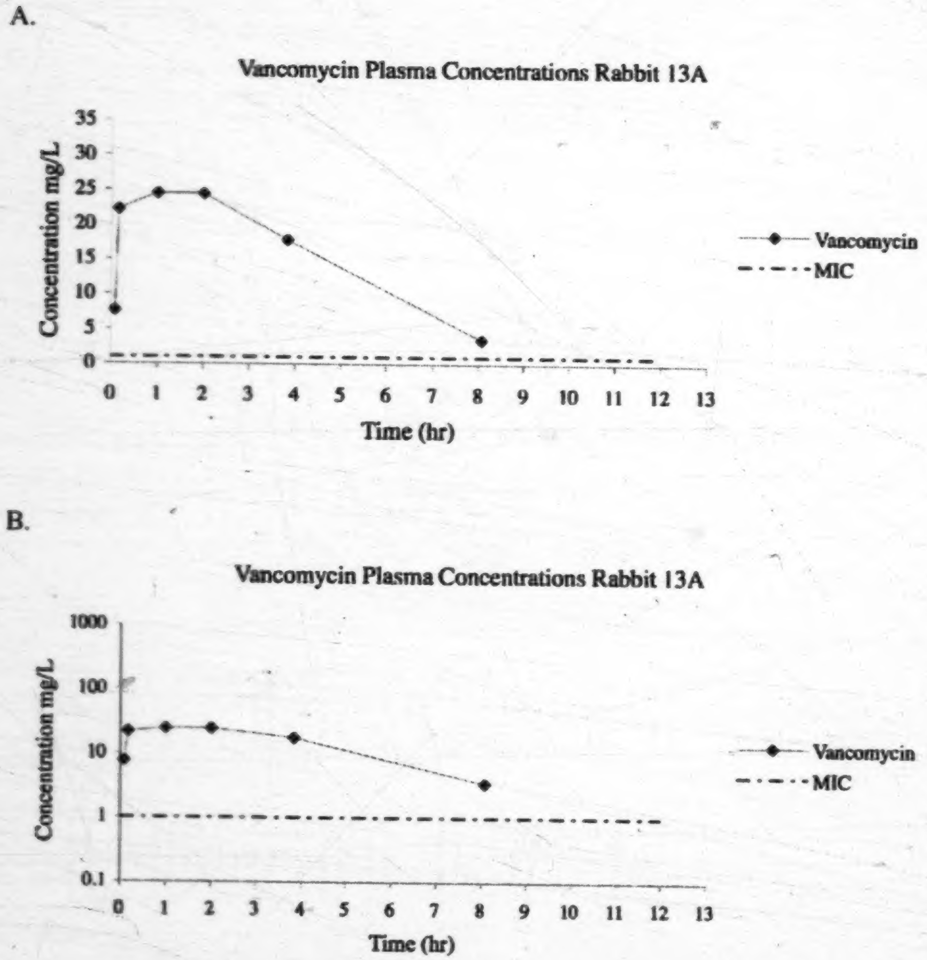


Figure 4.17. Vancomycin Plasma Concentrations Rabbit 13A.

Linear (A) and Log-linear (B) plots of plasma concentrations of vancomycin in Rabbit 13A following administration of vancomycin mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (1 μg/mL) between 8 and 12 hr of the 12 hr dosing interval at minimum. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory and do not appear on the graph.

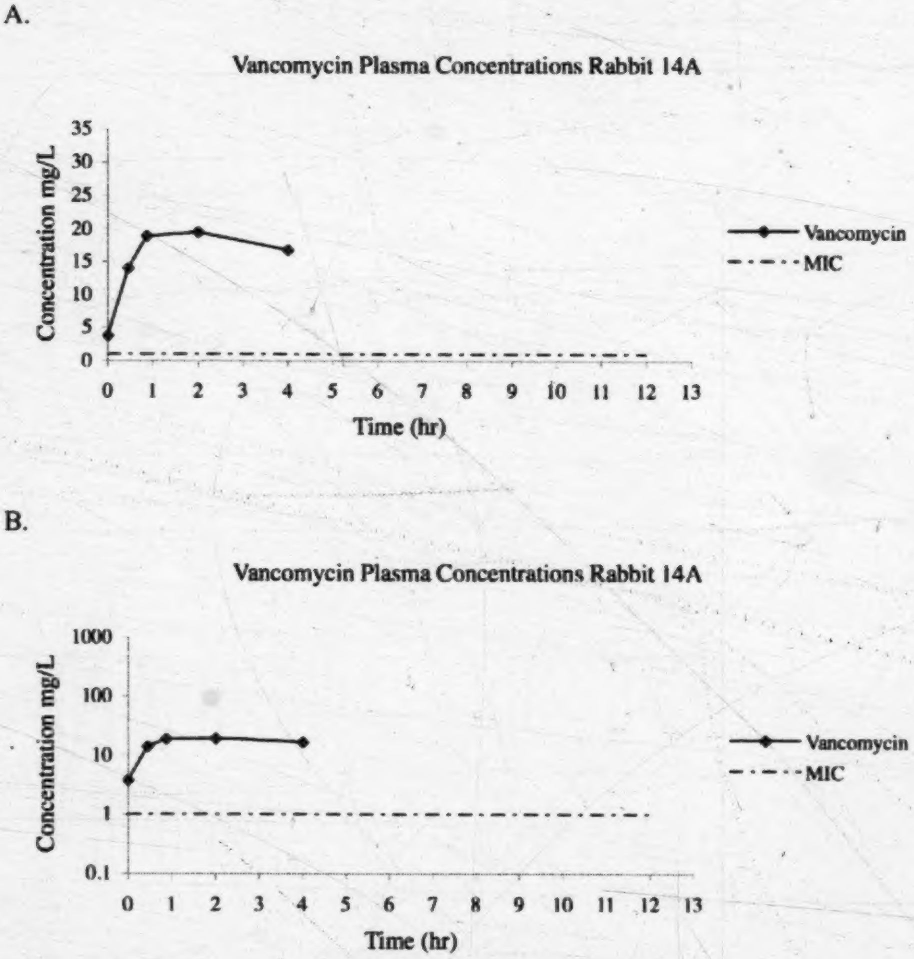


Figure 4.18. Vancomycin Plasma Concentrations Rabbit 14A.
Linear (A) and Log-linear (B) plots of plasma concentrations of vancomycin in Rabbit 14A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (1 μg/mL) at least 4 - 8 hr of the 12 hour dosing interval at minimum. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory and do not appear on the graph.

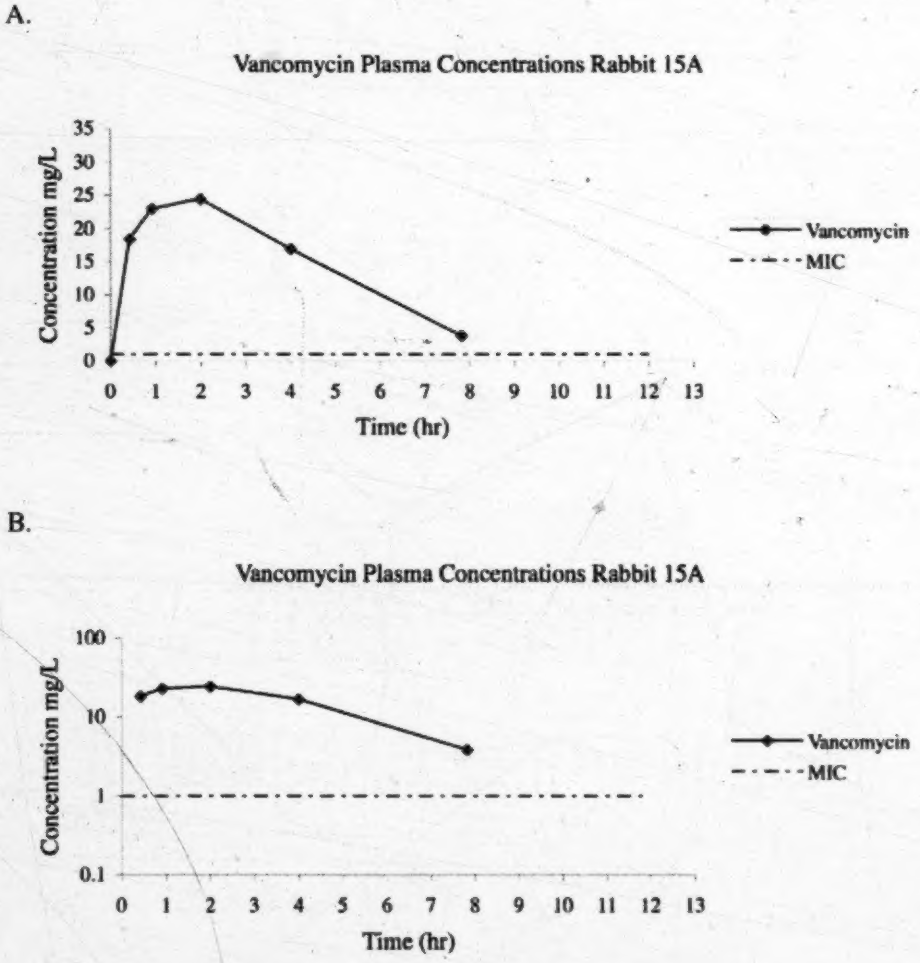
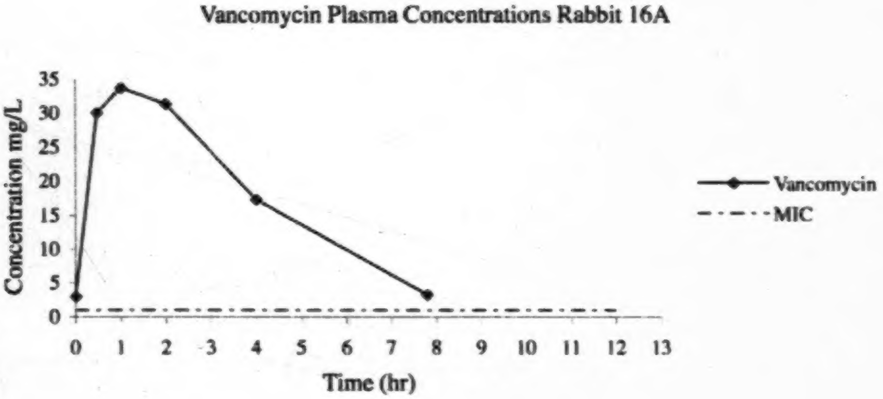


Figure 4.19. Vancomycin Plasma Concentrations Rabbit 15A.
Linear (A) and Log-linear (B) plots of plasma concentrations of vancomycin in Rabbit 15A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (1 μg/mL) between 8 and 12 hr of hr of the 12 hour dosing interval at minimum. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory and do not appear on the graph.

A.



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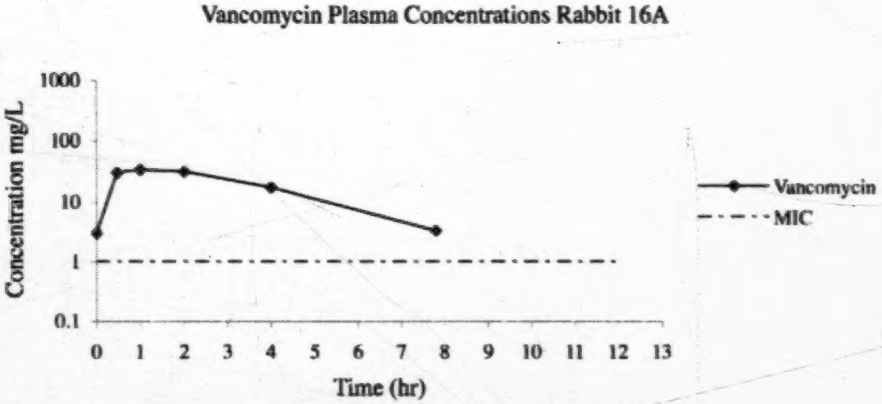


Figure 4.20. Vancomycin Plasma Concentrations Rabbit 16A.

Linear (A) and Log-linear (B) plots of plasma concentrations of vancomycin in Rabbit 16A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. Plasma concentrations remained above the MIC of *S. aureus* MU 7056 (1 μg/mL) between 8 and 12 hr of hr of the 12 hour dosing interval at minimum. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory and do not appear on the graph.

PDE Concentrations Vancomycin Rabbits 13A – 16A

Vancomycin concentrations in PDE for Rabbits 13A – 16A following 4 days of treatment with vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr with dialysis exchanges of 200 mL Dianeal 1.5% at 0, 4, 8 and 12 hr are shown in **Table 4.21**

Vancomycin concentrations were assayed at the University Hospital, Edmonton, Alberta. Concentrations above 100 μg/mL are reported form the laboratory as >100 mL and concentrations below 3 μg/mL are reported as < 3 μg/mL.

Maximum PDE concentrations were > 100 μg/mL in all rabbits with the first sample taken between 0.16 and 0.43 hr. Minimum concentrations were < 3 μg/mL in Rabbits 13A – 15A and 5.1 in Rabbit 16A. PDE concentrations of vancomycin remained above the MIC of vancomycin for *S. aureus* MU7056 of 1 μg/mL for 10 – 12 hr in all rabbits

Concentrations of vancomycin in PDE are shown in **Table 4.21** and Linear and Log-linear graphs of vancomycin concentrations in PDE over time are shown in **Figures 4.21 – 4.24.**

Table 4.21. PDE Vancomycin Concentrations.

PDE vancomycin concentrations are shown relative to the time of drug administration following administration of Vancomycin 20 mg/kg i.p. b.i.d. x 4 with peritoneal dialysis exchanges with 200 mL Dianeal 1.5% performed at 0, 4, 8 and 12 hours each day. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory and concentrations greater than 100 μg/mL were reported as 100 μg/mL.

Rabbit 13A		**Rabbit 14A**		**Rabbit 15A**		**Rabbit 16A**	
Time hr	PDE Concentration μg/ml	Time hr	PDE Concentration μg/ml	Time hr	PDE Concentration μg/ml	Time hr	PDE Concentration μg/ml
-0.08	<3	-0.13	23.1	-0.22	11.5	-0.12	24.7
0.16	100	0.4	100	0.38	100	0.43	100
0.92	100	0.92	100	1	100	0.99	100
1.96	100	1.92	100	2.92	100	2	97.6
3.92	61.7	3.89	100	3.95	81.7	3.99	38.8
7.92	5.3	7.95	15.2	8.0	7.9	11.88	5.1
12.0	<3	11.87	<3	11.92	<3		
23.3	6.3	23.5	13.5	22.9	<3		
T_{max}	0.16 hr	T_{max}	0.4hr	T_{max}	0.38hr	T_{max}	0.43hr
C_{max}	100 μg/ml	C_{max}	100 μg/ml	C_{max}	100 μg/ml	C_{max}	100 μg/ml

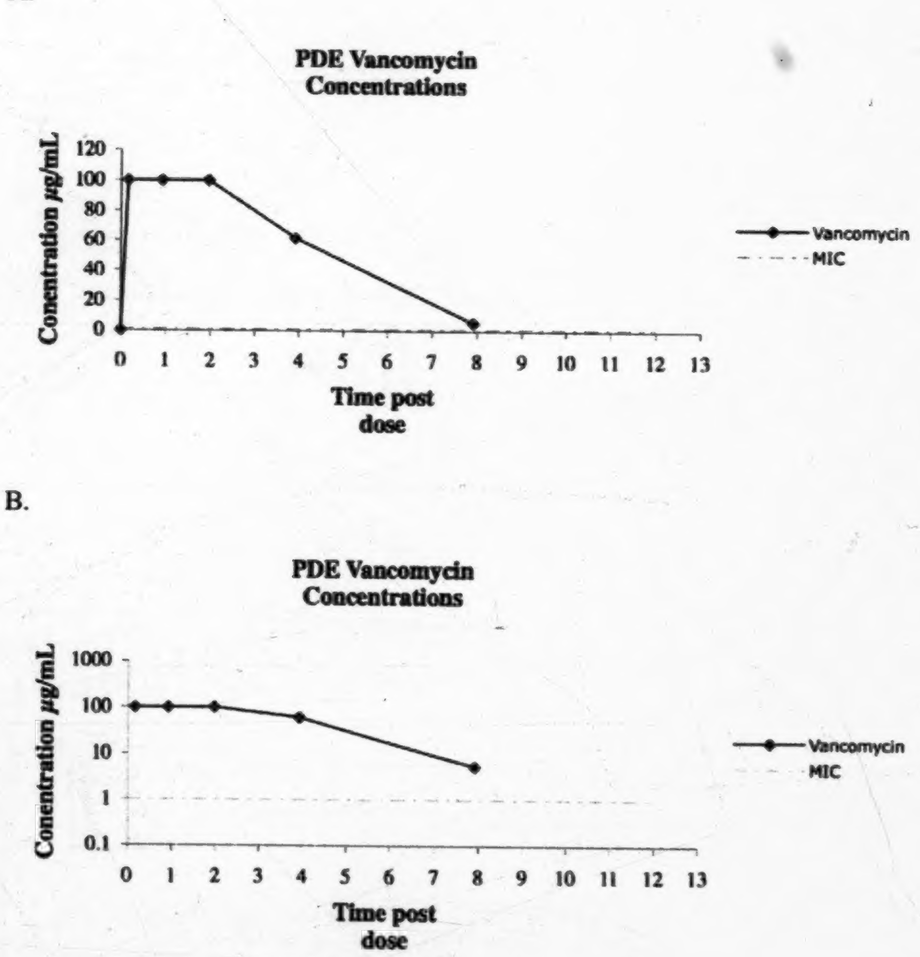
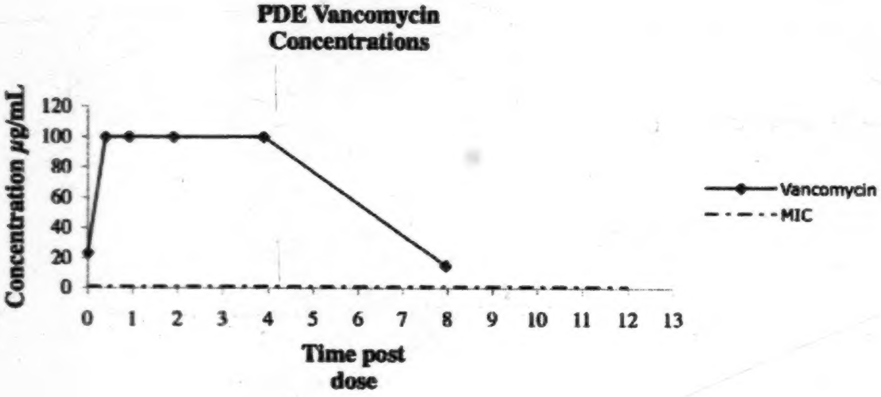


Figure 4.21. Concentrations of Vancomycin in PDE Rabbit 13A

Linear (A) and Log-linear (B) plots of PDE concentrations of vancomycin (µg/mL) in Rabbit 13A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (1 µg/mL) between 8 and 12 hr (approximately 10 hr extrapolated) of the 12 hour dosing interval at minimum. Concentrations above 100µg/mL were reported by the laboratory as >100 µg/mL and appear as 100 on the graph. Concentrations below 3 µg/mL were reported only as <3µg/mL by the laboratory and do not appear on the graph.

A.



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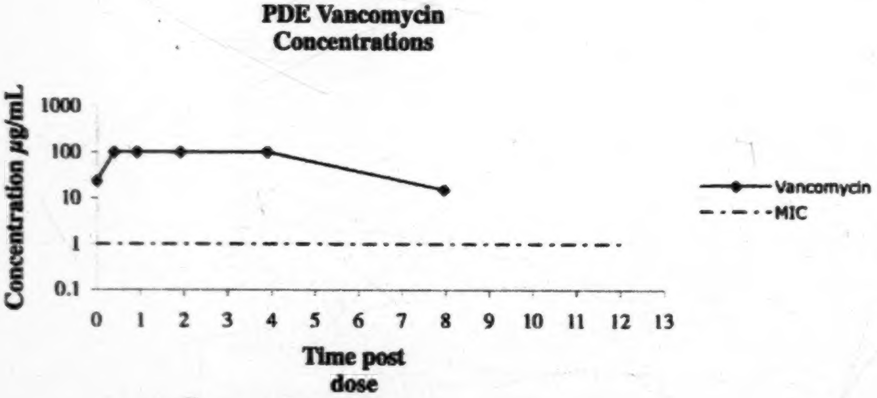
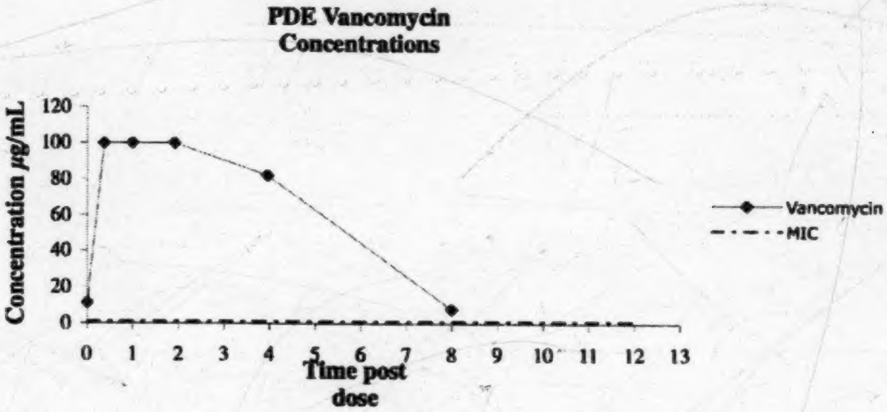


Figure 4.22. Concentrations of Vancomycin in PDE Rabbit 14A

Linear (A) and Log-linear (B) plots of PDE concentrations of vancomycin in Rabbit 14A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (1 µg/mL) between 8 and 12 hr (12 hr extrapolated) of the 12 hour dosing interval at minimum. Concentrations above 100µg/mL were reported by the laboratory as >100 µg/mL and appear as 100 on the graph. Concentrations below 3 µg/mL were reported only as <3µg/mL by the laboratory and do not appear on the graph.

A.



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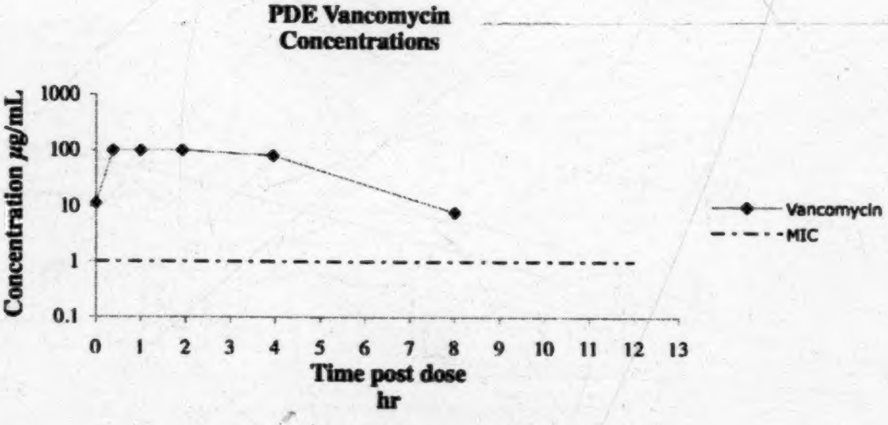

Figure 4.23. Concentrations of Vancomycin in PDE Rabbit 15A
Linear (A) and Log-linear (B) plots of PDE concentrations of vancomycin in Rabbit 15 A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours each day. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (1 μg/mL) between 8 and 12 hr (11 hr extrapolated) of the 12 hour dosing interval at minimum. Concentrations above 100μg/mL were reported by the laboratory as >100 μg/mL and appear as 100 on the graph. Concentrations below 3 μg/mL were reported only as <3μg/mL by the laboratory and do not appear on the graph.

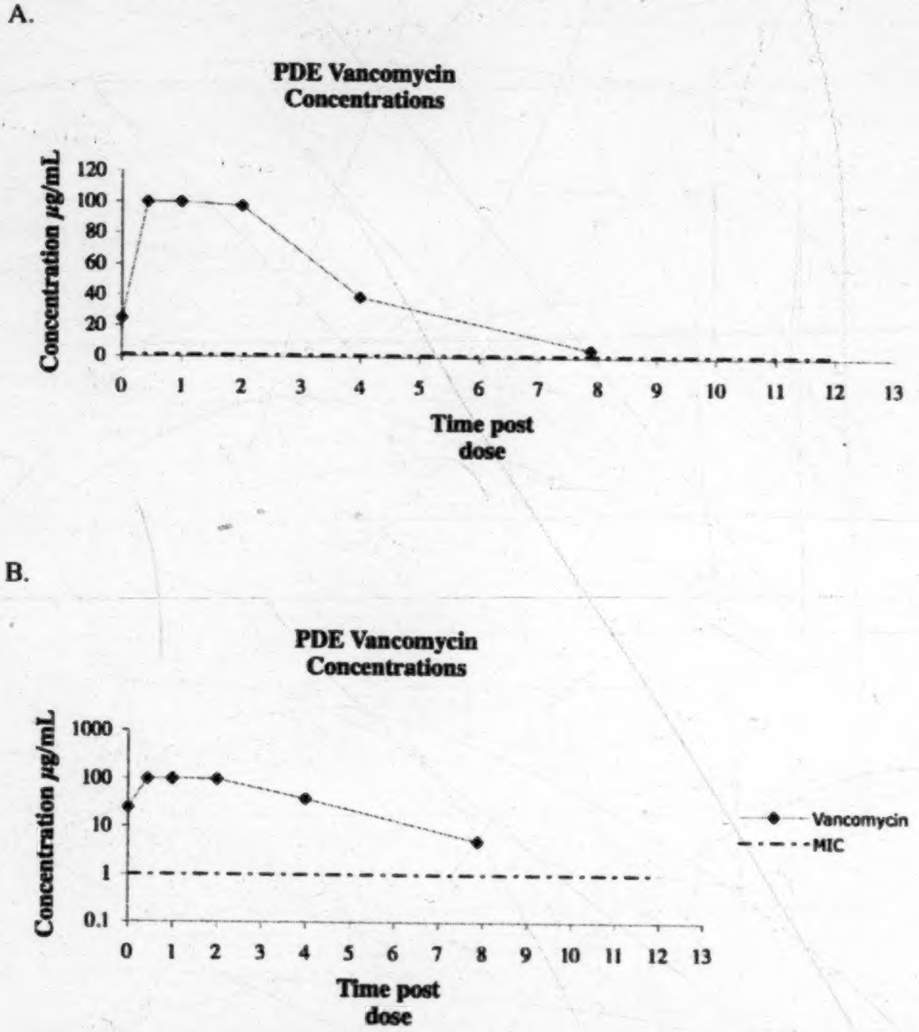

Figure 4.24. Concentrations of Vancomycin in PDE Rabbit 16A
Linear (A) and Log-linear (B) plots of PDE concentrations of vancomycin in Rabbit 16A following administration of vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days. Peritoneal dialysis exchanges with 200 mL Dianeal 1.5% were performed at 0, 4, 8 and 12 hours Day 10 and only twice a day Days 11 – 13 due to catheter failure. PDE concentrations remained above the MIC of *S. aureus* MU 7056 (1 µg/mL) for the entire dosing interval. Concentrations above 100µg/mL were reported by the laboratory as >100 µg/mL and appear as 100 on the graph. Concentrations below 3 µg/mL were reported only as <3µg/mL by the laboratory and do not appear on the graph.

Table 4.22. Plasma Vancomycin 24 hr AUC, AUC_{24}/MIC ratio, t>MIC and Extrapolated t> MIC

Plasma vancomycin 24 hr AUC, AUC_{24}/MIC ratio, t>MIC and extrapolated t> MIC following 4 days of treatment with vancomycin 20mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges each day.

	24 hr AUC Plasma μg/mL*hr	**24 hr AUC_{24}/MIC Plasma**	**t> MIC (hr) Plasma**	**Extrapolated t> MIC (hr) Plasma**
Rabbit 13A	271.40	271.40	> 8	10.5
Rabbit 14A	173.73	173.73	> 4	
Rabbit 15A	257.53	257.53	> 8	10.5
Rabbit 16A	302.92	302.92	> 8	10.5
Mean ± SE	251.40 ± 27.57	251.40 ± 27.57		

Table 4.23. PDE Vancomycin 24 hr AUC, AUC_{24}/MIC ratio, t>MIC and Extrapolated t> MIC

PDE vancomycin 24 hr AUC, AUC_{24}/MIC ratio, t>MIC and extrapolated t> MIC following 4 days of treatment with vancomycin 20mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges each day.

	24 hr AUC Plasma μg/mL*hr	**24 hr AUC_{24}/MIC PDE**	**t> MIC (hr) PDE**	**Extrapolated t> MIC (hr) PDE**
Rabbit 13A	991	991	> 8	10.5
Rabbit 14A	1291	1291	> 4	
Rabbit 15A	1155	1155	> 8	10.5
Rabbit 16A	868	868	> 8	10.5
Mean ± SE	1075 ± 92.6	1075.95 ± 92.6		

Table 4.24. **Plasma and PDE Concentrations of Vancomycin and D/P ratios**

Concentrations Vancomycin Rabbits 9-11 Day 4 (Thursday)															
Rabbit 13A	PD	Plasma	D/P	Rabbit 14A	PD	Plasma	D/P	Rabbit 15A	PD	Plasma	D/P	Rabbit 16A	PD	Plasma	D/P
Time Post	Conc.	Conc.	Ratio	Time Post	Conc.	Conc.	Ratio	Time Post	Conc.	Conc.	Ratio	Time Post	Conc.	Conc.	Ratio
Dose (hr)	µg/mL	µg/mL		Dose (hr)	µg/mL	µg/mL		Dose (hr)	µg/mL	µg/mL		Dose (hr)	µg/mL	µg/mL	
0.08	<3	7.7		-0.13	23.1	3.8	6.08	0.08	11.5	<3		0	24.7	3	8.23
0.16	100	22.2	4.5	0.45	100	14	7.14	0.42	100	18.4	5.43	0.47	100	30	3.33
1	100	24.5	4.08	0.87	100	18.9	5.29	0.92	100	23	4.35	1	100	33.7	2.97
2	100	24.4	4.1	2	100	19.5	5.13	2	100	24.4	4.1	2	97.6	31.4	3.11
3.83	61.7	17.8	3.5	4	100	16.8	5.95	4	81.7	16.9	4.83	4	38.8	17.3	2.24
8.08	5.3	3.5	1.51	8	15.2	<3		7.83	7.9	3.8	2.08	7.8		3.3	
11.83	<3	<3		12.3	<3	<3		11.45	<3	<3		11.7	5.1	<3	
23.3	6.3	<3		23.5	13.5	<3		22.9	<3	3.8					

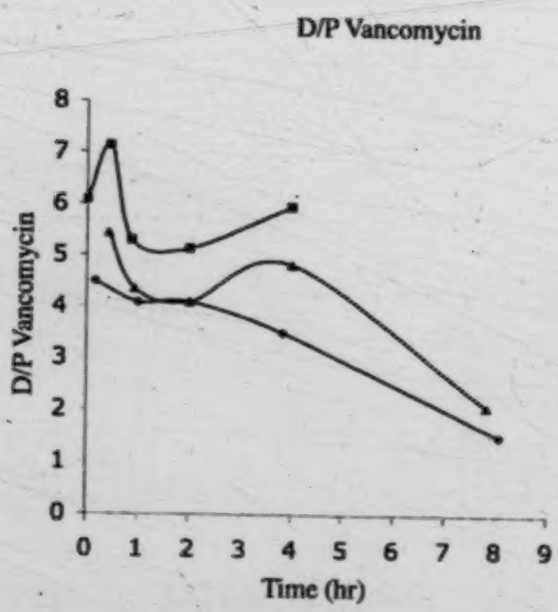

Figure 4.25. **D/P Ratios Vancomycin**

- The D/P ratio is indicative of peritoneal membrane permeability. Variability in D/P ratio with inflammation due to infection may account for some of the variability in the pharmacokinetic profile between rabbits.

Culture and S.E.M. Scans of Tissue and PD Catheter Samples – Vancomycin Group

Tissue and PD catheter sample cultures following treatment with vancomycin 20 mg/kg i.p. b.i.d. at 0 and 12 hr x 4 days with peritoneal dialysis exchanges performed at 0, 4, 8 and 12 hrs each day are shown as CFU/mL/g tissue or catheter in **Table 4.25.**

Rabbits 13A and 15A showed no bacterial growth at any site. In Rabbit 14A, cultures were positive for only the external catheter and the subcutaneous tunnel at 10 cm.

Rabbit 16A had positive cultures at 4 of the 7 sites, all identified as *S. aureus* MU7056. In this rabbit the catheter had malfunctioned and did not allow withdrawal of fluids. As a result rabbit 16A received vancomycin 20 mg/kg i.p. b.i.d. in 200 mL of Dianeal 1.5% but no fluid was withdrawn and further dialysis exchanges were not done.

Scanning Electron Microscopy of Internal and External Catheter Samples in Rabbits 13A - 16A Treated with Vancomycin.

Results of S.E.M. scans of the internal and external PD catheter samples are shown with correlated cultures in adjacent PD catheter samples in **Table 4.26.**

S.E.M. scans of the internal PD catheter samples correlated well with cultures. S.E.M. scans were negative in all rabbits except Rabbit 16A in which the S.E.M. was positive for cocci which correlated with a positive culture for *S. aureus MU7056.*

S.E.M. scans of the external catheter were negative in all rabbits despite bacterial growth in adjacent catheter samples in Rabbits 14A and 16A with *S. aureus* and *S. aureus* MU7056 respectively.

Table 4.25. Tissue / Catheter Sample Culture Results Vancomycin Treatment Group

Colony counts (CFU/g/mL) *S. aureus* MU7056 are shown correlated with bacterial identification pre- and post treatment with vancomycin 20 mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges daily with 200 mL Dianeal 1.5% x 4 days. *S. aureus* strains other than MU7056 (*S. aureus**), and coagulase negative *S. epidermidis* were cultured in addition to *S. aureus* MU7056 at 2 sites.

	Rabbit # 13A	**Rabbit # 14A**	**Rabbit # 15A**	**Rabbit # 16A**
Dacron Cuff	No growth	No growth	No growth	3.0×10^1
Internal Catheter	No growth	No growth	No growth	4.0×10^1
External Catheter	No growth	1.1×10^4 *S. aureus**	No growth	5.0×10^1
Peritoneal Wall Tissue	No growth	No growth	No growth	No growth
Peritoneal Wall Tissue	No growth	No growth	No growth	No growth
Subcutaneous Tunnel 10 cm	No growth	7.7×10^2 *S. aureus** CNS	No growth	2.8×10^1
Subcutaneous Tunnel 20 cm	No growth	No growth	No growth	No growth

Table 4.26. **Scanning Electron Microscopy of Internal and External Catheter Samples Rabbits 13A -16A Treated with Vancomycin .** S.E.M. and culture results of the internal and external catheter are shown correlated with bacterial identification pre- and post treatment with vancomycin 20 mg/kg i.p. b.i.d. in the first and last of 4 dialysis exchanges daily with 250 ml Dianeal 1.5% x 4 days. Bacterial strains isolated were identified as *S. aureus* MU7056 (S. A. MU7056). *S. aureus* strains other than MU&056 (*S. aureus**) were found in addition to *S. aureus* MU7056 at one site. S.E.M. results for the internal and external catheter are shown as negative, or positive for cocci or rods.

	Rabbit 13A	**Rabbit 14A**	**Rabbit 15A**	**Rabbit 16A**
S.E.M. Internal Catheter (Peritoneal Cavity)	Negative	Negative	Negative	Cocci
Culture Internal Catheter (Peritoneal Cavity)	No growth	No growth	No growth	4.0×10^{1}
S.E.M. External Catheter (Tunnel)	Negative	Negative	Negative	Negative
Culture External Catheter (Tunnel)	No growth	1.1×10^{4} *S. aureus**	No growth	5.0×10^{1}

Log_{10} Reduction CFU/mL in PDE in Vancomycin Treated Rabbits

Day 11, following inoculation Days 6, 7, and 8 and 2 dialysis treatments Day 10 to ensure development of peritonitis and before initiation of treatment with vancomycin, $\log_{10}$ bacterial CFU/mL in PDE ranged from 2.78 – 5.14 with a mean ± SE of 3.96 ± 0.68.

Day 15, following 4 days of treatment with vancomycin 20 mg/kg b.i.d. i.p. b.i.d. in the first and last of 4 dialysis exchanges each day with 250 ml Dianeal 1.5%, bacterial growth was not detected in PDE samples of rabbits 13A, 14A and 15A . PDE samples were not available for Rabbit 16A due to a malfunctioning catheter.

The $\log_{10}$ reduction of CFU/mL over the treatment period ranged from 2.78 – 5.14 with a mean ± SE of 3.96 ± 0.68. A 3 $\log_{10}$ reduction, is indicative of a 99.9% reduction in bacterial growth while a 4 $\log_{10}$ reduction is indicative of a 99.99% reduction in bacterial growth.

Log_{10} CFU/mL and $\log_{10}$ reductions in bacterial growth in PDE are reported for Rabbits 9-12 in **Table 4.27** while a bar graph of the mean $\log_{10}$ reduction ± SE in PDE in the vancomycin and SYN 1193 groups following 4 days of treatment are shown in **Figure 4.25.**

Time to 1 Log_{10} Reduction PDE Bacterial Growth in Vancomycin Group

The time required for a one $\log_{10}$ reduction in bacterial growth in PDE, a reflection of how quickly the antibiotic takes effect, was ≤ 1 day in Rabbits 13A and 15A and ≤ 2 days in Rabbit 14A (**Table 4.28**). Results were similar in the SYN 1193 group, the time required for a one $\log_{10}$ reduction in bacterial growth in PDE was ≤ 1 day in Rabbits 9 and 10 and ≤ 2 days in Rabbit 11 (**Table 2.29**).

Table 4.27. Log_{10} Reduction of CFU / mL in PDE of Vancomycin Treated Rabbits

Log_{10} bacterial colony counts in PDF Days 10, before treatment and day 15, the last day of treatment with Vancomycin 20mg/kg. PDE in rabbits 13A to 15A was completely cleared of bacterial growth. The bottom row shows the $\log_{10}$ reduction in CFU/mL over the treatment period. A 3 $\log_{10}$ reduction in indicative of a 99.9% reduction in bacterial growth while a 4 $\log_{10}$ reduction is indicative of a 99.99% reduction in bacterial growth.

	Rabbit 13A Log_{10} CFU/g/mL	**Rabbit 14A Log_{10} CFU/g/mL**	**Rabbit 15A Log_{10} CFU/g/mL**	**Rabbit 16 Log_{10} CFU/g/mL A**	**Mean ± SE Log_{10} CFU/g/mL**
Day 10	3.95	2.78	5.14	Catheter chewed	3.96 ± 0.68
Day 15	0	0	0		0
Log_{10} Reduction	**3.95**	**2.78**	**5.14**		**3.96 ± 0.68**

Efficacy - Treatment of Infection in PDE

All control rabbits showed bacterial growth $> 10^3$ CFU/mL with a mean ± SE of $9.8 \times 10^6 \pm 7.2 \times 10^6$ CFU/mL PDE. All control animals became very ill and were euthanised on compassionate grounds.

SYN 1193 completely cleared bacterial growth in PDE in Rabbits 9 and 11. PDE in Rabbit 10 had a colony count of 1.3×10^2 CFU/mL in PDE with a 2.11 $\log_{10}$ reduction in bacterial growth. The mean ± SE $\log_{10}$ reduction of CFU/mL in PDE in the SYN 1193 group Day 15 post-treatment relative to Day 11 pre-treatment was 4.34 ± 1.09.

PDE in all three vancomycin treated rabbits was completely cleared of bacterial growth. The mean ± SE $\log_{10}$ reduction of CFU/mL in PDE of the vancomycin group Day 15 post-treatment compared to Day 11 pre-treatment was 4.96 ± 0.68. The difference in $\log_{10}$ reduction in bacterial growth between the SYN 1193 and vancomycin groups was not significant $p > 0.9999$ (Mann-Whitney Test) as shown in **Figure 4.26**. The time to achieve 1 $\log_{10}$ reduction in bacterial growth was similar in the SYN 1193 and vancomycin treated groups ≤ 1 day in 2 rabbits and ≤ 2 days in 1 rabbit in each group **Tables 4.28** and **4.29**.

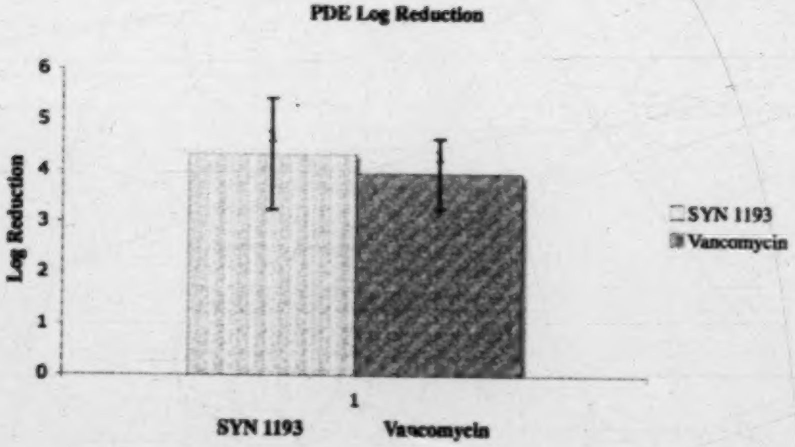

Figure 4.26. Log$_{10}$ Reduction of CFU/mL in PDE in SYN 1193 and Vancomycin Treated Rabbits

Log$_{10}$ reduction in bacterial colony counts were 4.34 ± 1.09 (mean ± SE) and 3.96 ± 0.68 on day 15, following 4 days of treatment with SYN 1193 or vancomycin 20 mg/kg b.i.d. i.p. in the first and last of four dialysis treatments each day respectively. This difference was not significant $p > 0.9999$ (Mann-Whitney Test).

Table 4.27. Log$_{10}$ Reduction of CFU / mL in PDE of Vancomycin Treated Rabbits

Log$_{10}$ bacterial colony counts in PDF Days 10, before treatment and day 15, the last day of treatment with Vancomycin 20mg/kg. PDE in rabbits 13A to 15A was completely cleared of bacterial growth. The bottom row shows the log$_{10}$ reduction in CFU/mL over the treatment period. A 3 log$_{10}$ reduction in indicative of a 99.9% reduction in bacterial growth while a 4 log$_{10}$ reduction is indicative of a 99.99% reduction in bacterial growth.

	Rabbit 13A	**Rabbit 14A**	**Rabbit 15A**	**Rabbit 16**	**Mean ± SE**
	Log$_{10}$ CFU/g/mL	**Log$_{10}$ CFU/g/mL**	**Log$_{10}$ CFU/g/mL**	**Log$_{10}$ CFU/g/mL A**	**Log$_{10}$ CFU/g/mL**
Day 10	3.95	2.78	5.14	Catheter chewed	3.96 ± 0.68
Day 15	0	0	0		0
Log$_{10}$ Reduction	**3.95**	**2.78**	**5.14**		**3.96 ± 0.68**

Table 4.28. **Time to One Log_{10} CFU/mL Reduction PDE Bacterial Growth in Vancomycin Treated Group**
Time to reduction of bacterial growth by 1 $\log_{10}$ was ≤ 2 days following administration of vancomycin 20 mg/kg i.p. q12h with the first and last of 4 dialysis exchanges with 250 ml Dianeal 1.5%.

	Rabbit 13A CFU/mL	**Rabbit 13A** Log_{10} CFU/mL	**Rabbit 14A** CFU/mL	**Rabbit 14A** Log_{10} CFU/mL	**Rabbit 15A** CFU/mL	**Rabbit 14A** Log_{10} CFU/mL	**Rabbit 16A** CFU/mL
Day 11	9.0×10^3	3.95	6.0×10^2	2.78	1.4×10^5	5.14	No sample
Day 12	2.7×10^2	2.43	9.0×10^1	1.95	3.0×10^2	2.48	No sample
Day 13	No Growth	No Growth	No Growth	No Growth	No Growth		No sample
Time to 1 Log_{10} CFU/g/mL Reduction		**≤1 day**		**≤2 day**		**<1 day**	

Efficacy – Treatment of Infection at Tissue / Catheter Sites

Control Rabbits 1-4

All control rabbits became very ill and were euthanised on compassionate grounds before the end of the study (Day 15). Rabbit 2 was euthanised Day 11 and Rabbits 1, 3 and 4 were euthanised Day 13. No rabbits in the SYN 1193 or vancomycin treated groups required euthanasia.

Bacterial cultures in tissue and catheter samples were positive in all but two sites in Control rabbits 1-4. CFUs/g/mL ranged from 1.3 x 10^2 to 7.0 x 10^7 at necropsy. Seven of the eight samples from the internal and external catheter were positive for the presence of biofilm on S.E.M. Rabbit 2 showed no growth from the two samples of peritoneal tissue and the subcutaneous tissue at 20 cm and a negative S.E.M. for adherent bacteria in the internal catheter. This rabbit was euthanised early on Day 11 on compassionate grounds.

Vancomycin Treated Rabbits 13A-16A

Tissue and catheter samples in the Rabbits 13A and 15A were completely cleared of bacterial growth post-treatment with vancomycin. Rabbit 14A showed bacterial growth in the external catheter of 1.1 x 10^4 CFU/g/mL and subcutaneous tunnel of 7.7 x 10^2 CFU/g/mL as shown in Table **4.29**. Seven of the eight samples from the internal and external catheter were negative for the presence of biofilm.

SYN 1193 Treated Rabbits 9-12

Rabbit 9, treated with SYN 1193, was completely cleared of bacterial growth at all tissue and catheter sites post-treatment. Rabbits 10 and 11 had positive cultures at 4 of the 8, and 6 of the 8 tissue/catheter sites respectively as shown in **Table 4.30**. Bacterial counts ranged from 4.5 x 10^1 to 4 x 10^5 and were markedly decreased compared to controls . S.E.M. was positive for the presence of adherent bacteria in 6 of the 8 samples of catheter as shown in **Table 4.30**. Representative scanning electron micrographs from the external catheter, internal catheter and Dacron cuff are shown in **Figures 4.27, 4.28, and 4.29** respectively.

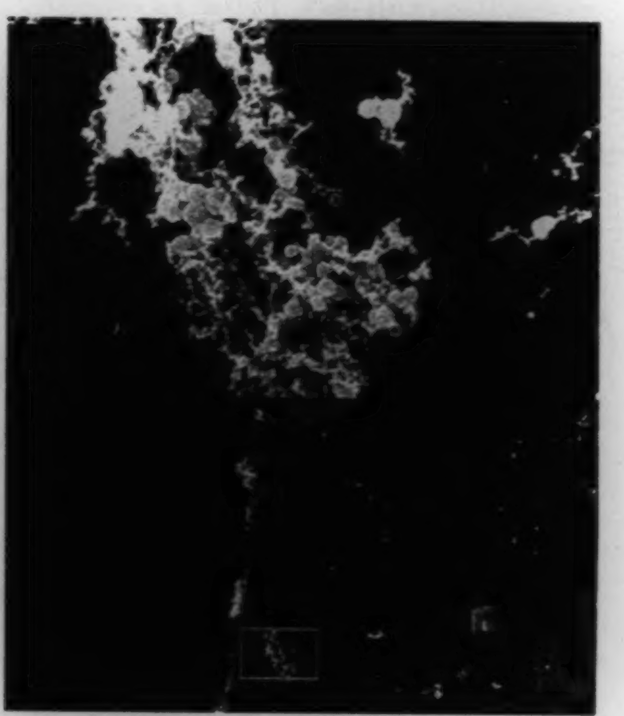

Figure 4.27. Representative S.E.M. from the External Catheter showing Biofilm.

Figure 4.28. Representative Scanning Electron Micrograph showing Biofilm on Internal Catheter

Figure 4.29. Representative S.E.M. of Biofilm Growth on Dacron Cuff

Tissue / Catheter Samples

Vancomycin treated rabbits showed a mean ± SE $\log_{10}$ reduction in bacterial CFU/g/mL for all tissue / catheter samples compared to controls of 4.98 ± 1.74, indicative > 99.99 % reduction in CFU/g/mL. Bacterial growth was diminished in the SYN 1193 group relative to Controls with a mean ± SD $\log_{10}$ reduction in bacterial growth of 3.85 ± 2.27 from all tissue / catheter sites indicative of a 99.99%

reduction in bacterial growth. Comparison of mean $\log_{10}$ reduction in all tissue samples is shown in **Figure 4.27**. No difference was found between the $\log_{10}$ reductions in the vancomycin and SYN 1193 groups $p>0.05$ (Mann-Whitney U Test).

Colony counts at each site and $\log_{10}$ reductions relative to controls are shown in **Table 4.29**. Mean bacterial counts (CFU/g/mL) for each site and number of rabbits showing bacterial growth at each site are shown in **Table 4.30**. Mean $\log_{10}$ reductions in bacterial growth of all tissue / catheter samples in the vancomycin and SYN 1193 groups are shown in **Figure 4.30**. Statistical analysis of individual sample sites with the Kruskall-Wallis test showed no significant difference in colony counts in 3 sites - the subcutaneous tunnel at 20 cm, and the 2 samples of peritoneal tissue $p > 0.05$. Significant differences were found in bacterial growth (CFU/g/mL) in samples from the Dacron cuff, internal and external catheter and subcutaneous tunnel at 10 cm $p < 0.05$. Dunn's post-hoc analysis showed significant differences in bacterial growth (CFU/g/mL) between groups at these sites. Significant reductions in bacterial growth were found in the vancomycin group compared to controls in samples obtained from the Dacron cuff $p< 0.01$, internal catheter $p< 0.05$ and external catheter $p< 0.05$. Significant reductions in bacterial growth were found in the SYN 1193 group compared to control in samples obtained from the internal catheter and subcutaneous tunnel at 10cm $p< 0.05$. No differences were found between the effect of the SYN 1193 and vancomycin on bacterial growth at any site $p> 0.05$.

Table 4.29. Tissue / Catheter Sample Results Colony counts (CFU/g/mL) of tissues and $\log_{10}$ –reduction in CFU/g/mL post-treatment with SYN 1193 and vancomycin relative to controls.

SYN 1193	Rabbit 9	Rabbit 10	Rabbit 11	Rabbit 12	Mean R9-11	S.D. R 9-11	S.E. R 9-11	Log_{10} Reduction R9-11	
Dacron Cuff	0.00E+00	4.10E+05	4.60E+05	6.00E+01	2.90E+05	2.52E+05	1.46E+05	1.98E+00	
Internal Catheter	0.00E+00	0	2.30E+02	0	7.67E+01	1.33E+02	7.67E+01	5.48E+00	
External Catheter	0.00E+00	1.10E+02	6.00E+01	4.90E+02	5.67E+01	5.51E+01	3.18E+01	5.51E+00	
Peritoneum	0.00E+00	5.90E+03	5.60E+01	0	1.99E+03	3.39E+03	1.96E+03	4.43E-01	
Peritoneum	0.00E+00	0	1.50E+02	0.00E+00	5.00E+01	8.66E+01	5.00E+01	2.13E+00	
Subcutaneous Tunnel 10 cm	0.00E+00	0	0.00E+00	6.60E+02	0.00E+00	0.00E+00	0.00E+00	6.26E+00	
Subcutaneous Tunnel 20 cm	0.00E+00	0	0.00E+00	4.50E+01	0.00E+00	0.00E+00	0.00E+00	5.12E+00	
Mean	0.00E+00	5.94E+04	6.58E+04	1.79E+02				Mean 3.85E+00	SD 2.27E+00

Vancomycin	Rabbit 13A	Rabbit 14A	Rabbit 15A	Rabbit 16A	Mean R13A-16A	S.D. R13A-16A	S.E. R13A-16A	Log_{10} Reduction R9-11	
Dacron Cuff	0.00E+00	0.00E+00	0.00E+00	3.00E+01	0.00E+00	0.00E+00	0.00E+00	7.45E+00	
Internal Catheter	0.00E+00	0.00E+00	0.00E+00	4.00E+01	0.00E+00	0.00E+00	0.00E+00	7.37E+00	
External Catheter	0.00E+00	1.10E+04	0.00E+00	5.00E+01	3.67E+03	6.35E+03	3.67E+03	3.46E+00	
Peritoneum	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	3.74E+00	
Peritoneum	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	3.83E+00	
Subcutaneous Tunnel 10 cm	0.00E+00	7.70E+02	0.00E+00	2.80E+01	2.57E+02	4.45E+02	2.57E+02	3.85E+00	
Subcutaneous Tunnel 20 cm	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	5.12E+00	
Mean	0.00E+00	1.68E+03	0.00E+00					Mean 4.98E+00	SD 1.74E+00

Control	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Mean R1-4	S.D. R1-4	S.E. R1-4
Dacron Cuff	1.20E+07	2.10E+07	2.90E+07	5.00E+07	2.80E+07	1.62E+07	8.11E+06
Internal Catheter	2.30E+07	1.80E+05	8.40E+04	7.00E+07	2.33E+07	3.29E+07	1.65E+07
External Catheter	8.10E+05	4.00E+05	7.10E+07	1.60E+06	1.85E+07	3.50E+07	1.75E+07
Peritoneum	1.40E+04	0.00E+00	6.30E+03	1.70E+03	5.50E+03	6.26E+03	3.13E+03
Peritoneum	8.10E+03	0.00E+00	1.90E+04	1.30E+02	6.81E+03	8.97E+03	4.48E+03
Subcutaneous Tunnel 10 cm	9.20E+04	2.70E+03	7.20E+06	1.60E+04	1.83E+06	3.58E+06	1.79E+06
Subcutaneous Tunnel 20 cm	4.30E+05	0.00E+00	1.10E+04	9.10E+04	1.33E+05	2.02E+05	1.01E+05
Mean	5.19E+06	3.08E+06	1.53E+07	1.74E+07	1.02E+07	7.16E+06	3.58E+06

Table 4.30. Comparison Residual Infection in Tissue/Catheter Sites with Vancomycin, SYN 1993 and Controls

Total number of rabbits in each treatment group showing residual infection with mean CFU/g/mL at each tissue/catheter sample site with results of Kruskall Wallis Test with Dunn's Multiple Comparisons Post-hoc analysis and Mann Whitney U Test (C=control, S = SYN 1193 V = vancomycin.) No statistical difference between vancomycin and SYN 1193 treatment was found at any site.

	Positive Cultures				
	Control Rabbits 1-4	**SYN 1193 Rabbits 9-11**	**Vancomycin Rabbits 13A-15A**	**Kruskall Wallis/ Dunn's potshot test**	**Mann Whitney U**
Dacron Cuff	4 / 4 2.8 x10^7	2 / 3 2.9 x10^5	0 / 3 0	p = 0.0119 Css >0.05 CvsV **<0.01** SvsV >0.05	CvsS **0.029** CvsV **0.029** SvsV 0.14
Internal Catheter	4 / 4 2.3 x10^7	1 / 3 7.7 x10^1	0 / 3 0	p = 0.0119 CvsS **<0.05** CvsV **<0.05** SvsV >0.05	CvsS **0.029** CvsV **0.029** SvsV 0.143
External Catheter	4 / 4 1.85 x10^7	2 / 3 5.7 x10^1	1 / 3 3.7 x10^3	p = 0.0198 CvsS >0.05 CvsV **<0.05** SvsV >0.05	CvsS **0.029** CvsV **0.029** SvsV 0.099
Subcutaneous Tunnel 10cm	4 / 4 1.83 x 10^6	0 / 3 0	1 / 3 2.6 x10^7	p = 0.014 CvsS **<0.05** CvsV NS SvsV NS	CvsS **0.029** CvsV **0.029** SvsV 0.47
Subcutaneous Tunnel 20 cm	3 / 4 1.33x 10^5	0 / 3 0	0 / 3 0	p=0.0585	CvsS NS CvsV SvsV
Peritoneal Wall	3 / 4 5.5 x 10^3	2 / 3 1.9 x10^3	0 / 3 0	p=0.15	CvsS CvsV SvsV
Peritoneal Wall	3 / 4 6.81 x 10^3	1 / 3 5.0x10^1	0 / 3 0	p=0.085	CvsS NS CvsV SvsV

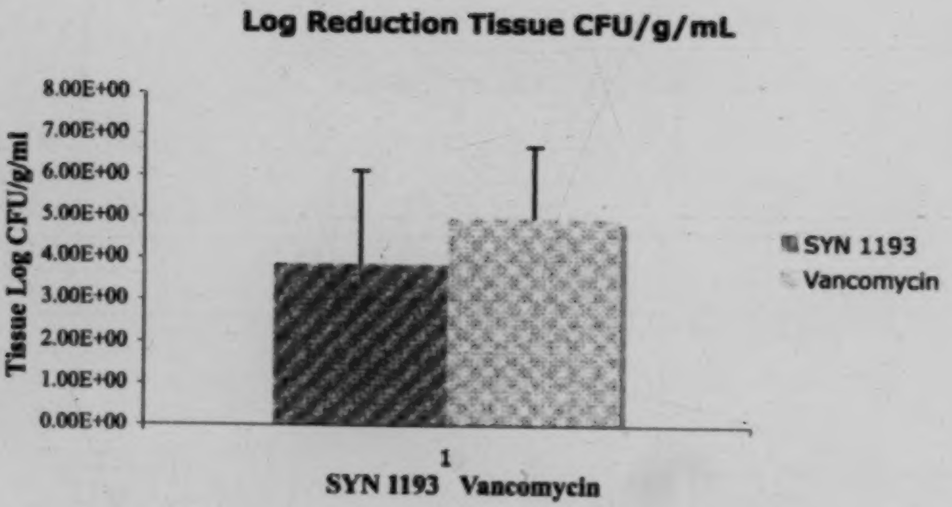

Figure 4.30. **Mean $\log_{10}$ reduction bacterial growth (CFU/g/mL) in all tissue and catheter samples in SYN 1193 and Vancomycin Treated Groups.** (peritoneal tissues, subcutaneous tissues and internal and external catheter and Dacron cuff). Treatment with SYN 1193 resulted in 3.85 ± 2.27 $\log_{10}$ reduction in bacterial growth while treatment with vancomycin resulted in a 4.98 ± 1.74 $\log_{10}$ reduction in bacteria growth. A 3 $\log_{10}$ reduction in CFUs is indicative of a 99.9% reduction in bacterial growth and a 4 $\log_{10}$ reduction indicative of a 99.99% reduction in bacterial growth. There was no statistical difference between the SYN 1193 group and the vancomycin group $p>0.05$.

Discussion

Pharmacodynamics - Rabbits Treated with SYN 1193

In our study, SYN 1193 20 mg/kg q12h administered i.p. diffused well across the peritoneal membrane along concentration gradients. Administration of SYN 1193 via the i.p route resulted in high initial PDE concentrations, with peaks ranging from 89.8-115.7 μg/mL, while plasma concentrations are low. The drug diffused rapidly across the peritoneal membrane into plasma resulting in peak concentrations in plasma of 8.15 – 12.9 μg/mL by 0.42 – 1 hr. Peak concentrations were approximately 10% of those achieved in PDE. Conversely, as SYN 1193 is removed from the peritoneal cavity during dialysis exchanges at 4 and 8 hrs, PDE concentrations fall below those in plasma and SYN 1193 diffuses back into the peritoneal cavity to maintain adequate concentrations of SYN 1193 in the PDE throughout the 12 hour dosing interval despite dialysis exchanges. This is shown in Rabbit 10 after the 8 hr dialysis exchanges at 4 and 8 hr in **Figure 4.12.** Protein binding likely contributes somewhat to the difference in peak concentrations between PDE and plasma protein binding of SYN 1193 (approximately 80% in plasma in rabbits). Only the unbound drug would cross the peritoneal membrane. The volume of distribution of SYN 1193 would likely affect the plasma concentration, e.g., the greater the volume of distribution, the lower concentration in plasma, to a greater extent.

Almost complete absorption of SYN 1193 occurs by 4 hours before the first dialysis exchange assuming minimal binding to peritoneal tissues and the silicone catheter.

In the treatment of peritonitis in PD patients, it is very important that adequate concentrations of antibiotic are maintained in both the peritoneal cavity and the plasma. Adequate concentrations of antibiotic are required in the peritoneal cavity to treat the local manifestations of peritonitis, infection of the peritoneal fluid, the mesothelial layer of the peritoneal membrane and the catheter within the peritoneal cavity. Biofilm may form on the catheter in the peritoneal cavity or the peritoneal membrane resulting in infection more resistant to treatment. Adequate concentrations of antibiotic are also required in the plasma to treat infection that tracks along the subcutaneous tunnel surrounding the catheter and infection along the surfaces of the catheter imbedded in biofilm in the subcutaneous tunnel (9, 103).

Peak/MIC Ratios SYN 1193 in PDE and Plasma

Intraperitoneal dosing of 20 mg/kg SYN 1193 q12h resulted in high concentrations in PDE with peak concentrations ranging from 89.8 μg/mL to 115.7 μg/mL with a mean ± SE of 104.03 ± 7.59. Resultant peak/MIC ratios in PDE ranged from 179.6 to 231.4 with a mean ± SE of 208.06 ± 15.17. Considering protein binding of SYN 1193 of 80% in rabbits a free peak/MIC ratio of 41.61 ± 3.03 would be well in excess of the peak/MIC ratio of 8 – 20 reported to correlate with efficacy and the prevention of development of resistance.

SYN 1193 20mg/kg administered i.p. q12h appeared to diffuse well across the peritoneal membrane resulting in peak plasma concentrations of 8.15 – 12.9 μg/mL by 0.42 – 1 hr. Resultant total plasma peak/MIC ratios of SYN 1193 were 16.3 - 25.8 μg/mL. Although total peak/MIC ratios would easily reach the goal of 8 – 20, free peak/MIC ratios of 3.26 – 5.16 would be inadequate.

Total peak/MIC ratios achieved in PDE and plasma exceed the values of 10 - 20 found to be predictive of successful therapy as well of those of 8 – 10 μg/mL (44) (42) associated with the development of resistance. Free peak/MIC ratios would be adequate in PDE but not adequate in plasma.

AUC_{24}/MIC Ratios SYN 1193 in PDE and Plasma

The AUC_{24} of SYN 1193 achieved in PDE in this rabbit study ranged from 153.24 – 216.98 µg/mL*hr with a mean of 181.87 ±18.68. Resultant PDE total AUC_{24}/MIC ratios were 306.48 – 350.78. Free AUC_{24}/MIC ratios in PDE ranged from 61.3 – 70.16 falling within the range of 50 ± 20 and 81± 37 correlated with efficacy for treatment of *S. aureus* by Andes [Andes, 2003 #748] and Craig (32). The total AUC_{24}/MIC ratios in PDE exceeded the AUC_{24}/MIC ratio of approximately 100 required for prevention of the development of resistance (42, 55), but free concentrations did not. The original study was reported using total concentrations, however the higher degree of protein binding with SYN 1193 relative to ciprofloxacin in the original study (80% vs. 35%) reduces the free fraction. The findings in this study suggest that the dose of SYN 1193, 20 mg/kg i.p. q12h used in this study provides adequate drug exposure in PDE for the treatment of planktonic bacteria in peritonitis despite dialysis exchanges but is unlikely to be high enough for the prevention of the development of resistance. It is unknown if the residual bacterial growth in PDE Rabbit 11 after 4 days treatment with SYN 1193 is due to the development of resistance during therapy, reseeding of infection from biofilm on catheter or tissues, or the abbreviated 4 day treatment. There was, however, a > 2 $\log_{10}$ reduction in bacterial growth in this rabbit.

The 24 hr AUC in plasma ranged from 34.42 – 70.40 µg/mL*hr with a mean ± SE of 48.39 ± 7.79 µg/mL*hr. Plasma AUC_{24}/MIC ratios ranged from 94.27 – 140.80 with a mean ± SE of 96.57 ± 15.67. Free AUC_{24}/MIC ratios ranged from 18.85 – 28.16 with a mean ± SE of 19.31 ± 3.13. The free AUC_{24}/MIC ratios attained in plasma do not reach the target of free AUC_{24}/MICs ratio of 50 ± 20 and 81 ± 37 reported by Craig and Andes (51, 52) to correlate with successful eradication of *S. aureus*.

If total concentrations are considered, the AUC_{24}/MIC ratio is within the range of the AUC_{24}/MIC ratio required for prevention of development of resistance. The original studies reporting this AUC_{24}/MIC ratio of 100 used HPLC assays and reported total concentrations. Although a number of antibiotics were used in these patients, ciprofloxacin was the fluoroquinolone used(55). The higher degree of protein binding with SYN 1193 relative to ciprofloxacin (80% vs. 35%) makes it unlikely that drug exposure is adequate to prevent the development of resistance.

It is difficult to know if this drug exposure would be adequate to eradicate the bacteria trapped in biofilm on the catheter surface. Concentrations in PDE may be adequate but concentrations in plasma would likely not be adequate. In the past few years, in vitro work has been done by Ceri, Olson, Sepandj et al. to determine the concentrations of antibiotics required to kill bacteria within a biofilm, termed the minimum biofilm eradication concentration (MBEC) (99, 101, 102). The MBEC of SYN 1193 against *S. aureus* MU7056 is not known. MBECs for various organisms have been shown to range from concentrations identical to MICs, to concentrations 500 fold or more times the MIC. It is interesting to note that in a study done by Olson et al. with veterinary pathogenic strains, the MIC of enrofloxacin, a fluoroquinolone, was the same as the MBEC when tested against one strain of *E. coli*, however the MIC was <2 μg/mL and MBEC 128 μg/mL with another strain. In this study, enrofloxacin was the only antibiotic effective against *P. aeruginosa* in the sessile form although several were effective against the planktonic form. It is not known if this activity against biofilm extends to other fluoroquinolones or to activity against biofilm formed by *S. aureus*. Dasgupta et al. studied the activity of erythromycin and two fluoroquinolones, SYN 1193, and sparfloxacin against biofilm formed by methicillin-resistant *S. aureus* using a modified Robbins device. (25). In this study, SYN 1193 was found to have the highest biofilm bacterial killing effect against MRSA. Neither sparfloxacin nor erythromycin resulted in significantly greater efficacy than placebo. Although erythromycin had been shown previously to be effective in the destruction of

Pseudomonas biofilms, it was not effective alone against MRSA biofilm in this study, and when tested in combination with SYN 1193 or sparfloxacin, unfortunately, erythromycin did not alter he biofilm permeability and killing of the quinolones for MRSA biofilms (25).

Studies by Firsov, evaluating mutant prevention concentrations of four quinolones, moxifloxacin, levofloxacin, ciprofloxacin and gatifloxacin against MRSA, showed that there was no change in MIC following treatment if AUC_{24}/MIC values of 201-244 were achieved. Similar studies by Firsov with ABT 492 found AUC_{24}/MIC ratios of 240 and 200 to be protective against mutant selection with *S. aureus*. Using this criteria, an AUC_{24}/MIC of 96.57 ± 15.67 in plasma would not be adequate for prevention of mutant selection and enhancement. The AUC_{24}/MIC ratio of 363.74 in PDE would be adequate for prevention of mutant selection and enhancement. The AUC_{24} of 181.8±18.68 achieved in PDE would prevent mutation selection in stains with MICs < 0.76.

Studies of garenoxacin by Tam et al., reported that an AUC_{24}/MIC ratio of 67 was required for the prevention of selection of resistant mutants against MSSA, an AUC_{24}/MIC ratio of 144 for ciprofloxacin-sensitive, MRSA and an AUC_{24}/MIC of > 447 for ciprofloxacin MRSA *(59)*. These findings raise some concerns regarding the adequacy of coverage for prevention of mutant selection in our rabbit studies with *S. aureus* MU7056, a ciprofloxacin-resistant strain of MRSA.

According to the findings of Tam et al (2001), a 24 hr PDE AUC of 181.87, found in our studies, would be high enough to prevent selection of resistant mutants with strains of ciprofloxacin-sensitive MSSA with MICs less than 181.68/67 = 2.71 μg/mL, and ciprofloxacin–sensitive MRSA with MICs up 181.68/144 = 1.26 μg/mL. In these rabbit studies with ciprofloxacin-resistant MRSA MU7056, the dose of SYN 1193 20 mg/kg i.p. q 12h with peritoneal dialysis exchanges at 4 and 8 hours would only provide adequate AUC_{24}/MIC ratio for prevention of mutant selection with strains with MICs < 0.41 μg/mL SYN 1193 (the MIC of *S. aureus* MU7056 is 0,5μg/mL SYN 1993).

Similarly, the 24 hr AUC of 48.39 ±7.79 achieved in plasma in these studies, would only be high enough to prevent selection of resistant mutants in strains of ciprofloxacin-sensitive MSSA with MICs less than 48.39/67 = 0.72 μg/mL, ciprofloxacin –sensitive MRSA with MICs up to 48.39/144 = 0.33μg/mL and ciprofloxacin resistant strains such as *S. aureus* MU7056 with MICs less than 48.39/447= 0.11.

The dose of SYN 1193 could be adjusted or given with each dialysis exchange to attain a higher AUC_{24}/MIC ratio with a higher cutoff MIC for all strains of *S. aureus*. Alternatively an oral dose could be given in conjunction with an i.p. dose. One also must take into consideration that the half-life of drugs is often much shorter in small animals than humans, therefore, dosing in humans may lead to AUC_{24}/MIC ratios necessary for prevention of mutation selection (107). One must caution that the area of study of MPC and prevention of mutation selection is in its infancy. This study by Tam et al (2001) is the one in vitro study that has looked at the MPC for ciprofloxacin-resistant MRSA that has only been reported in abstract form to date. Pharmacodynamic parameters may prove to be different when studied in an in vivo environment with an intact immune system.

Percent Time Concentrations Greater Than MIC (%t> MIC)

Most studies to date have not found %t>MIC to be the most important factor in predicting efficacy of microbial kill or clinical treatment with the fluoroquinolone antibiotics. However, in the first clinical study of ciprofloxacin, Peloquin et al. found that %t > MIC was the most important factor in predicting clinical efficacy (36). With the addition of 24 patients and reanalysis of the patients from the Peloquin study, Forrest et al. determined AUC_{24}/MIC to be most important in determining efficacy (37). Animal studies using the mouse thigh model by Craig and Andes (51) delineating the pharmacodynamic parameters associated with successful eradication of *S. aureus, S. pneumoniae and Enterobacteraciae* with gatifloxacin showed that the PKPD parameter that best correlated with efficacy was AUC_{24}/MIC (R^2=90-94%). The correlation with peak/MIC was 70-81% and %t>MIC 48-73%. In a study with garenoxacin by Andes and Craig,(52) the results were similar. The correlation of PKPD parameters with efficacy were AUC_{24}/MIC ratio (R^2 = 71-90%), peak/MIC ratio (R^2 = 47 – 75%) and %t>MIC (R^2= 47 - 75%). Due to the interdependence of all of the three pharmacodynamic parameters, AUC_{24}/MIC ratio, peak/MIC ratio and %t>MIC, it is not surprising that all three factors would have some bearing on efficacy. Most in vitro or animal studies are done using dose fractionation to try and determine which PKPD parameter is most highly correlated with efficacy. For example, the same total dose may be given, but the dose will be given at varying intervals or the peak may be held constant varying the t1/2 and t>MIC. In vitro studies of bacterial killing of PRSP with 6 fluoroquinolones by Hershberger and Ryback, found that an AUC_{24}/MIC ratio of ≤40 or a %t> MIC of < 55% were associated with decreased killing and significant bacterial regrowth (45). Landmark clinical studies by Schentag et al. determined that an AUC_{24}/MIC ratio (AUIC) of >125 correlated with therapeutic efficacy with the fluoroquinolones. An AUC_{24}/MIC ratio of >125 approximates 80% of the dosing interval above the MIC (108).

Although %t>MIC is not the most important factor in determining efficacy with the fluoroquinolone/quinolone antibiotics, it is clear from these studies that it does play a role. Pharmacodynamic studies have not been done to simulate the situation found with peritoneal dialysis where the antibiotic is removed from PDE regularly and concentrations may fall below the MIC of the organism prematurely despite an adequate AUC_{24}/MIC ratio due to a high initial peak. Fluoroquinolones demonstrate a significant post-antibiotic effect, but this generally only lasts 1.5 - 2.5

hrs in gram-positive and gram-negative organisms (35) although post-antibiotic effects of 1 – 6 hours have been reported for the newer fluoroquinolones (27). If concentrations fall below the MIC for a prolonged period of time, the PAE may not be able to fill the gap. Under these circumstances it is wise to consider the %t>MIC to ensure bacterial re-growth does not occur. Duration of post-antibiotic effect may not be the only factor that must be considered in determining the time interval that drug concentrations may fall below the MIC without loss of efficacy. For example, it is well accepted that concentrations of ß-lactam antibiotics that are known to have short PAEs, must only remain above the MIC of the organism for approximately 50% of the dosing interval.

In Rabbits 9-11, PDE concentrations of SYN 1193 remained above the MIC for 9.5-10.5 hrs of the 12 hr dosing interval resulting in %t>MIC of 79.2 - 87.5 %. This exceeds the 55% finding of Hershberger and correlates well with the findings of Schentag, where a %t> MIC $\geq$ 80% and AUC_{24}/MIC ratio of $\geq$ 125 is predictive of efficacy and prevention of selective pressure for overgrowth of resistant sub-populations (109).

In plasma, concentrations of SYN 1193 for Rabbits 9-11 were maintained above the MIC of *S. aureus* MU7056 for 6-12 hr resulting in %t>MIC of 50-100%. This approximates the %t>MIC of > 55% described by Hershberger et al. It is interesting to note that in Rabbit 9, plasma concentrations were only maintained above the MIC for 6 hours (50% of the 12 hr dosing interval) yet cultures in PDE and tissues were all negative at necropsy. In Rabbit 12, the PD catheter had been blocked to withdrawal of fluid and so drug was administered q12h but could not be withdrawn. Dialysis exchanges were not done at 4 and 8 hours and PDE was not available for analysis. It is surprising that SYN 1193 concentrations in plasma only remained above the MIC of *S. aureus* for 3.5 hr or 29.2% of the dosing interval. On necropsy significant amounts of fibrin and fibrinous or sclerosed tissue were found to be blocking the catheter.

Once protein binding is taken into consideration, the t> MIC ranges from only 3.5 – 5.5 hr in PDE and 2-2.5 hr in plasma of Rabbits 9-11. If the target is > 55% of the dosing interval, these values fall short. These estimates assume that the protein concentration in PDE is the same as albumin which is an overestimate, providing the most conservative estimate of free drug concentrations in PDE. Graphs of free and total concentrations of SYN 1193 in PDE and plasma are shown in **Figures 4.31 – 4.33**

Preliminary in vitro work has been done to determine the application of MPC in prevention of mutant selection and enhancement throughout the dosing interval. AUC_{24}/MICs as previously discussed have been correlated with mutant selection and enhancement. Campion et al., using simulated pharmacokinetic patterns to examine the effect ciprofloxacin with ciprofloxacin-sensitive strains of *S. aureus*, found that ciprofloxacin concentrations entirely above the MPC appeared to eradicate low-level resistant variants and prevent the emergence of higher levels of resistance (67). Although the goal of our study, was not to study MPCs and emergence of resistance, it is clear that SYN 1193 concentrations did not remain above the MIC, and certainly not the MPC, for the entire dosing interval and the selection and enhancement of mutant strain would be a risk with this dosing regimen. Research in the area of mutant selection prevention is quite new. Studies have not been done to determine whether the antibiotic concentrations must remain above the MIC for the entire dosing interval or if there is a specific percent of time that a dosing regimen must stay above the MPC to prevent mutant selection and enhancement.

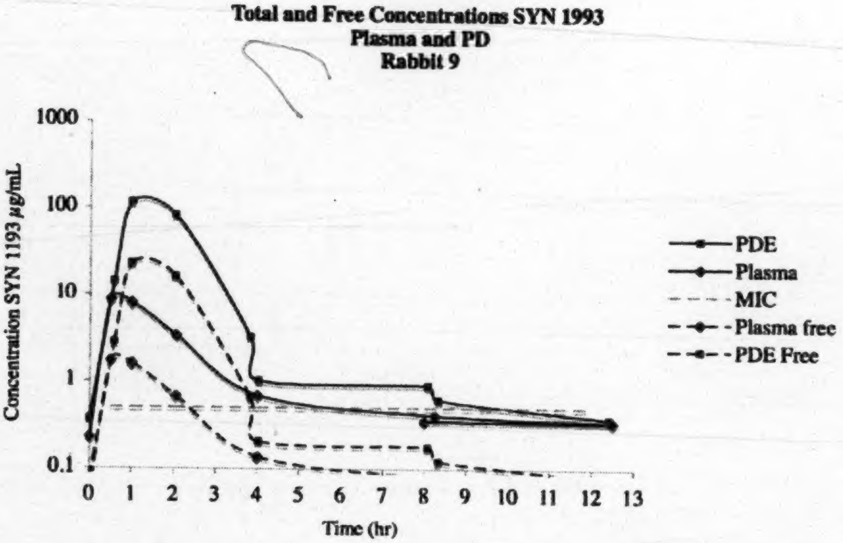

Figure 4.31. **Total and Free Plasma and PDE Concentrations SYN 1193 Rabbit 9.**

Plasma free concentrations of SYN 1193 remain above the MIC for 2.5 hr while PDE fluid concentrations remain above the MIC for 5 hr.

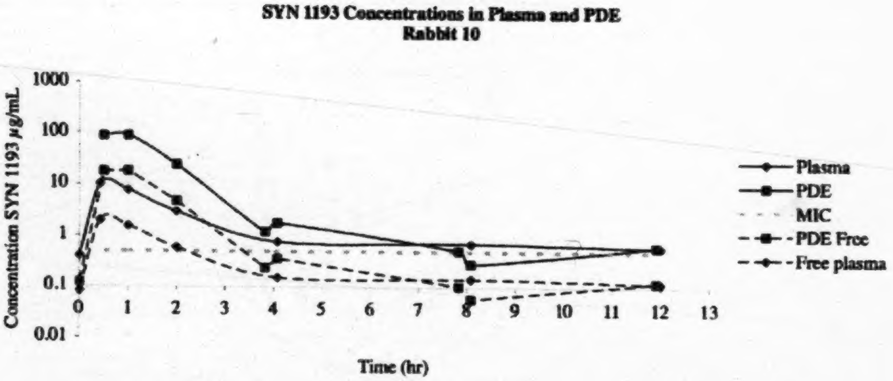

Figure 4.32. **Total and Free Plasma and PDE Concentrations SYN 1193 in Rabbit 10.** Plasma free concentrations of SYN 1193 remain above the MIC for approximately 2 hr while PDE fluid concentrations remain above the MIC for 3.5 hr.

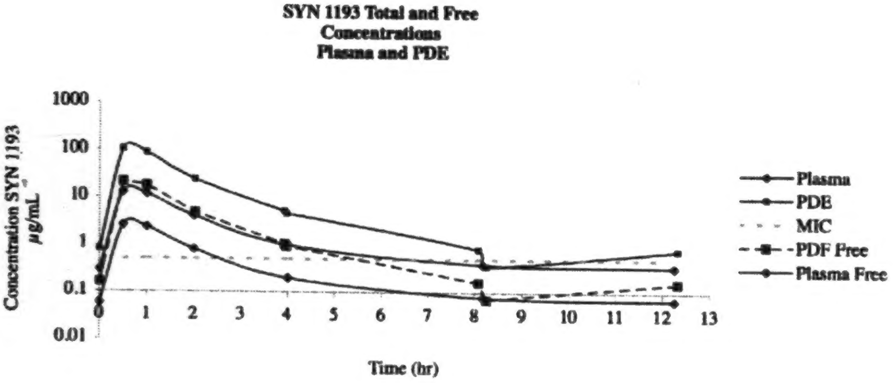

Figure 4.33. **Total and Free Plasma and PDE Concentrations SYN 1193 - Rabbit 11.**

Plasma free concentrations SYN 1193 remain above the MIC for approximately 2.5 hr while PDE fluid concentrations remain above the MIC for 5.5 hr.

Correlation of Plasma and PDE Concentrations SYN 1193

We have found a direct correlation between plasma and PDE concentrations. SYN 1193 is absorbed rapidly from the peritoneal cavity. Drug transfers along a concentration gradient initially from the peritoneal cavity to plasma when concentrations are high in the peritoneal cavity. Following dialysis, concentrations of SYN 1193 are higher in plasma resulting in diffusion across the peritoneal membrane into the peritoneal cavity. This is advantageous in this situation as concentrations above the MIC are maintained in the peritoneal cavity despite dialysis exchanges at 4 and 8 hours. An excellent correlation was found between plasma and PDE in rabbits 9-11, $R^2 = 0.74$, $p < 0.0001$ indicating that at any time, the concentration in the plasma reflects the concentration in the PDE.

Pharmacodynamic Targets - Rabbits 13A-16A Receiving Vancomycin

Peak Vancomycin Concentrations in PDE and Plasma

Rabbits 13A – 16A were given vancomycin 20 mg/kg i.p. q12h with dialysis exchanges at 0 and 12 hr with administration of vancomycin and at 4 and 8 hours. Peak concentrations achieved in PDE were in excess of 100 μg/mL. These concentrations are well in excess of the accepted therapeutic peak concentrations of 20 – 40 μg/mL. The MIC of vancomycin for *S. aureus* MU7056 was 1 μg/mL with resultant PDE peak/MIC ratios in excess of 100 μg/mL, well in excess of the free peak/MIC ratios of 5-6 reported by Knudsen et al. to correlate with efficacy [Knudsen, 2000 #35]. Vancomycin crossed the peritoneal membrane to plasma with plasma Cmax plasma concentrations ranging from 24.4 – 33.7 μg/mL. Estimating the free fraction of vancomycin to be 60%, free Cmax/MIC ranging from 14.64 – 20.22, would be well in excess of the free Cmax/MIC ratios of 5 - 6 reported by Knudsen et al. By all measures, the peak concentrations attained with this dosing regimen should be adequate to treat planktonic bacterial infection in the peritoneal cavity as well as infection in the subcutaneous tunnel. Results of colony counts in PDE and tissue / catheter samples concur with this.

Vancomycin AUC_{24}/MIC ratios in PDE and Plasma

The 24 hr AUC in PDE ranged from 867.81-1290.54 μg/mL*hr with AUC_{24}/MIC ratio of 867.81-1290.54 with the MIC of vancomycin of 1μg/mL for *S. aureus* MU7057. These are well in excess of the AUC_{24}/MIC ratios of 350 and 400 reported by Moise-Broder to correlate with successful treatment of pneumonia and the AUC_{24}/MIC ratio of 80 reported by Pavie to correlate with efficacy in the rabbit model of endocarditis as well as the AUC_{24}/MIC ratios of 86-460 reported by Dudley to be associated with 50% maximal killing in an in vitro model.

The 24 hr AUC in plasma ranged from 173.73 – 302.92 μg/mL*hr with AUC_{24}/MIC ratio of 173.73 – 302.92. These are well in excess of the AUC_{24}/MIC ratio of 80 reported by Pavie to correlate with efficacy in the rabbit model of *S. aureus* endocarditis and within the range of the AUC_{24}/MIC ratios of 86-460 reported by Dudley to be associated with 50% maximal killing using an in vitro model. The AUC_{24}/MIC ratios of173.73 – 302.92 do not quite meet the AUC_{24}/MIC ratios of 350 – 400 reported by Moise-Broder, however, as previously discussed, these studies were in patients with pneumonia and vancomycin concentrations in ELF are very low.

Microbiologic Efficacy

All control rabbits developed infection in the peritoneal fluid by day 6 with bacterial counts ranging from 2 x 10^1 to those too numerous to count. Day 7 bacterial counts ranged from 5.6 x 10^5 – 1.6 x 10^8. All control rabbits became very ill and were euthanised on compassionate grounds by Day 11 (Rabbit 2) or Day 13 (Rabbits 1, 3 and 4). At necropsy, PDE of all control animals were infected with colony counts ranging from 4.6 x 10^3 – 3.1 x 10^7. Of the four control animals *S. aureus* MU7056 was identified in all rabbits. Coliforms and CNS were identified as contaminants in the PDE of 3 rabbits. *S. aureus* MU 7056 was identified at all but 3 or the 28 tissue / catheter sites.

At necropsy, all control rabbits had positive cultures along the catheter, and subcutaneous tunnel. All but rabbit 2 had positive cultures of the peritoneal wall tissue. Some variability is to be expected due to natural variability in immune response. *S. aureus* MU7056 was identified at all sites along the catheter tract in all Rabbits indicating success with infection of these rabbits with this human strain of *S. aureus*. Rabbit 2 showed negative cultures in both samples of the peritoneal wall tissue and the sample of subcutaneous tissue at 20 cm. Scanning electron micrographs of the internal and external catheter samples were positive for the presence of biofilm on all but the internal catheter of Rabbit 2. The lack of infection of the peritoneal wall and the negative S.E.M. for adherent bacteria on the internal catheter of Rabbit 2 may be due to the necessity to euthanised this rabbit early on Day 11, the day after dialysis is performed to spread infection to the peritoneal cavity or it may be due to the natural variation in immune function and the patchy nature of the development of biofilm.

From these results, it appears that the rabbit model functioned as expected with the progression of infection from the exit site along the subcutaneous tract, through the Dacron cuff and into the peritoneal cavity, with development of peritonitis and biofilm along the catheter tract. Considering the fresh wound at the opening of the catheter, and the conditions of housing in a rabbit cage, it is not surprising that some organisms other than *S. aureus* MU7056 were found at some point along the route of infection due to bacterial contaminants colonizing the exit site and tracking along the catheter tunnel.

Survival

Although a very crude measure of efficacy of treatment, all of the control animals became very ill and were euthanised on compassionate grounds before the end of the study. None of the animals in the SYN 1193 or vancomycin groups had to be euthanised before the end of the study although they appeared to be ill at initiation of treatment and improved with treatment.

Efficacy in Treatment of Infection in PDE

I.P administration of antibiotics delivers high concentrations of antibiotic directly to the peritoneal cavity, the site of acute infection. In addition, high concentrations of antibiotic in PDE bathe the intraperitoneal membranes and intraperitoneal surfaces of the catheter are advantageous in the treatment of planktonic and sessile forms of bacterial infection.

At necropsy, PDE of all control animals was infected with colony counts ranging from 4.6 x 10^3 – 3.1 x 10^7 CFU/mL. All control animals required euthanasia before the end of the study, Rabbit 2 on Day 11 and Rabbits 1, 3 and 4 on Day 13.

SYN 1193 was very effective at eliminating or reducing planktonic bacterial growth in PDE. Rabbits 9-12 all showed extensive bacterial growth in PDE on Day 11 before antibacterial treatment was initiated with colony counts ranging from 2 x 10^4 to 1 x 10^6 CFU/mL. At necropsy, following 4 days of treatment, Rabbits 9 and 10 had completely eliminated all bacterial growth in PDE. Rabbit 11 showed markedly reduced growth in PDE of 1.3 x 10^2 CFU/mL with a 2.19 $\log_{10}$ (99%), reduction in CFU/mL. It is not known if the remaining bacterial growth in Rabbit 11 would respond to more prolonged treatment e.g. 14 -21 days as in the usual clinical treatment regimen. It is not known if this continued bacteria growth is due to the shorter, four day treatment regimen, protein binding, the development of resistant organisms during therapy, or the recurrence of infection in PDE due to reseeding of PDE from biofilm along the catheter tract. Culture of the external catheter in Rabbit 11 was positive for *S. aureus* MU7056 and on S.E.M., the internal catheter in Rabbit 11 was reported as ± cocci so reseeding form biofilm is a possibility.

Vancomycin completely cleared all bacterial growth in PDE of Rabbits 13A -15A after four days treatment with vancomycin. All rabbits showed bacterial growth in PDE ranging from 6 x 10^2 – 1.4 x 10^5 CFU/mL on Day 11 just before treatment was initiated. The catheter in Rabbit 16A had malfunctioned and we were unable to obtain a sample.

Log_{10} reductions CFU/ml in PDE were 4.34 ± 1.09 in the SYN 1193 group and 3.96 ± 0.68 in the vancomycin treated group after 4 days of treatment. Statistical analysis using the Mann Whitney U test showed no differences between these two groups $p > 0.05$ although the numbers in each group are small. The slightly lower $\log_{10}$ reduction in the vancomycin group is due, at least in part, to lower pre-treatment CFU/mL in PDE in the vancomycin group compared to the SYN 1193 group Day 11. However, vancomycin treatment completely eradicated all planktonic bacterial growth in PDE.

Rabbit 12 receiving SYN 1193 and Rabbit 16A receiving vancomycin were not evaluated because the PD catheters of both rabbits were blocked to withdrawal and we were unable to continue proper dialysis or take proper samples. Regular dialysis exchanges alone have been shown to be beneficial in the reduction of PDE colony counts in peritonitis(4), therefore, these rabbits would not provide a clear indication of the rate clearance of bacterial growth from PDE.

Tissue / Catheter Samples

Samples from the Dacron cuff, internal and external catheter, subcutaneous tunnel and peritoneum were cultured. Catheter surfaces were scraped with a sterile scalpel and sonicated to allow culture of adherent bacterial cells. In addition, adjacent catheter samples were examined using scanning electron microscopy to determine the presence of adherent bacterial biofilm.

Tissue / Catheter Results in Rabbits Treated with Vancomycin

All tissue / catheter samples from Rabbits 13A and 15A treated with vancomycin were negative for bacterial growth as were the S.E.M.s of these tissue and catheter samples. In Rabbit 14A, bacterial cultures of 2 sites, the external catheter and subcutaneous tunnel at 10 cm were positive, 1.1×10^4 CFU/mL and 7.7×10^2

CFU/mL. This sample was also positive for the presence of adherent bacteria on scanning electron microscopy while all other samples were negative. The overall $\log_{10}$ reduction of the average CFU/g/mL of all tissue sites following 4 days of treatment with vancomycin was 4.98 ± 1.74 relative to control rabbits indicative of close to > 99.999% bacterial killing.

After only 4 days of therapy, vancomycin 20 mg/kg/i.p. q12h was very effective in treating infection caused not only by planktonic but also sessile forms of bacteria in PDE tissue and on catheter surfaces. It is not known if this treatment continued for 14 – 21 days, as would occur in clinical practice, would eradicate any remaining organisms, but it is very likely that it would. Average initial CFU/mL in PDE was much higher in the SYN 1193 group than the vancomycin group, 2.43×10^5 vs. 3.13×10^3. This may be indicative of higher initial bacterial counts along the catheter surfaces, subcutaneous tissues and peritoneum as well in the SYN 1193 group that may contribute to some of the differences in response.

Tissue/Catheter Results in Rabbits Treated with SYN 1193

Rabbit 9 treated with SYN 1193 showed complete clearing of bacterial growth in all tissue and catheter samples and the SEM was negative for the presence of adherent bacterial in the internal catheter, however, the SEM was positive for the presence of adherent cocci in the external catheter within the tunnel. Bacterial growth was positive in three sites in Rabbit 10 and 5 sites in Rabbit 11 with mean bacterial CFU/g/mL for all sites of 5.94×10^4 and 6.58×10^4 respectively. The overall $\log_{10}$ reduction of all tissue sites was 3.85 ± 2.27 relative to control rabbits, indicative of > 99.9% bacterial killing.

Bacterial growth following treatment was found to be significantly reduced in the vancomycin group compared to the control group in the Dacron cuff, $p<0.01$, internal catheter $p<0.05$, and external catheter $p<0.05$ and in the SYN 1193 group in internal catheter and subcutaneous tunnel at 10 cm with the Kruskall Wallis Test with Dunn's Multiple Comparisons Post-hoc Analysis. The SYN 1193 and vancomycin groups were not statistically different at any site. **Table 4.30**

The results of S.E.M.s showed cocci in the external catheter of rabbit 9 and internal catheter of Rabbit 10 despite negative cultures, reinforcing the concern that although the planktonic forms of the bacteria may be easily treated, the sessile forms embedded in biofilm may persist, are much more difficult to eradicate, and may be resistant to antibiotic treatment providing a nidus for continued or recurrent infection.

With total clearing of PDE and all tissue / catheter samples on culture and only one segment of the catheter showing evidence of adherent cocci on SEM in Rabbit 9 after only 4 days of treatment, SYN 1193 shows promise in the treatment of peritonitis and associated catheter and tissue infections in PD patients with *S. aureus* infections including those that are methicillin and ciprofloxacin – resistant. Persistent bacterial growth and evidence of biofilm at 3 sites n Rabbit 10 and 5 sites in rabbit 11 are cause for concern even though bacterial growth is much less that in controls. Continuing treatment for 14 - 21 days, as would be given clinically, would be required to prove or disprove this hypothesis. Further studies, optimizing the free peak/MIC ratio and free AUC_{24}/MIC of SYN 1193 using increased doses i.p. or adding an oral dose in addition to the i.p. dose. In the study developing this rabbit model of catheter induced peritonitis in peritoneal dialysis, Read et al. were able to continue dialysis for 3 weeks (103) so this would be possible. Development of resistance during treatment may also play a role in the failure of response. Future studies should take this into consideration with determination of MIC in residual pathogens. Biofilm plays an important role in treatment failure and more research is required into the application of MPC and MBEC.

It may be possible to increase the dose of SYN 1193 given i.p. to achieve adequate free peak/MIC ratios of >8 and improved free AUC/MIC ratios in plasma. From toxicologic studies done by Synphar, the non-toxic dose of SYN 1193 was determined to be 25 mg/kg in rats and 10 mg/kg in dogs, however, these data do not show plasma concentrations achieved or the route of administration in these studies. This dose was not likely given by the i.p. route into peritoneal dialysate and it may well be possible to increase the i.p. dose without risk of toxicity considering the peak plasma concentrations achieved with i.p. administration are only approximately10% of those in PDE. Having access to the toxic plasma concentrations from toxicity studies done previously would confirm the maximum target concentration in plasma.

Induction of Renal Failure in the Rabbit Model

Rabbits used in this study did not have renal failure. Induction of renal failure may be advantageous. With induction of renal failure, excretion of drugs normally excreted renally such as vancomycin would be impeded and the half-life prolonged as in PD patients. In addition, induction of renal failure would result in accumulation of waste products that may compete for albumin binding sites and affect protein binding of drugs. Partial nephrectomy has been used to induce renal failure in animal models and could be used in the future with this model (110).

Half-lives of drugs are shorter in many animals than in humans, especially with small rodents. For example, the half-life of cefazolin is 15 minutes in mice and 108 minutes in humans. The administration of uranyl nitrate extends the $t_{1/2}$ of the drug by inducing transient acute tubular injury and the half-life becomes closer to that in humans (111). For example, with administration of uranyl nitrate, the $t_{1/2}$ of amikacin increased from 18 to 93 minutes in mice(111). The $t_{1/2}$ of amikacin in humans is approximately 104 minutes (111). This is particularly advantageous when trying to determine if extended interval dosing would be effective.

Induction of renal failure through partial nephrectomy would have little influence on the excretion of SYN 1193 since it is primarily excreted through the biliary tract with minimal amounts (0.4 – 7.6%) excreted renally in this rabbit model.

In normal patients, vancomycin is excreted primarily (80-90%) unchanged in the urine. Protein binding of vancomycin has been reported to be 25% in mice (85) and variably reported in humans to be 35-65%(85) (112) (113) (84) and 10 – 50% (114). Plasma concentrations of vancomycin and free concentrations would be higher in renal failure due to the effect of renal function.
For both SYN 1193 and vancomycin, treating the rabbits without inducing renal failure provides the most conservative estimate of efficacy of the antibiotic at any particular dose. Integrating specific pharmacodynamic targets with efficacy outcomes such as bacterial cultures and SEM for biofilm identification provides a much clearer picture as to the prospects of treatment success, particularly with the fluoroquinolone antibiotics. The ideal pharmacodynamic parameters correlated with efficacy of vancomycin treatment are quite controversial with several different types of studies reporting varying results. To date, no true dose fractionation studies have been done to definitively delineate the optimal pharmacodynamic parameters to ensure maximal efficacy of vancomycin treatment.

Future Approaches in the Study of Treatment of Peritonitis and Biofilm in PD

To prove definitive efficacy of SYN 1193 and vancomycin in the treatment of planktonic and sessile forms of bacterial infection, further animal studies should be done. Increasing the total duration of treatment to 14 -21 days in this rabbit model as would be done in clinical practice would allow us to determine if planktonic and sessile forms of bacteria were eradicated at all sites. Increasing the dose of SYN

1193 or adding an oral dose to ensure adequate free concentrations in plasma would ensure maximal benefit from SYN 1193 treatment. The dose of vancomycin could also be increased in future studies increasing trough concentrations to 10 – 15 mg/mL (79, 80) however from the results of this study that does not appear to be necessary – extending the treatment regimen to 14 -21 days would answer this question. Observing the animals for a period of time post-treatment would allow for the study of recurrence or relapse post-treatment. There is concern regarding the development of resistance and selection of mutant strains during therapy. Future studies should include the determination of MIC of any residual bacteria post treatment to ensure that this was not occurring during treatment. In the future, this model could be used along with induction of renal failure by partial nephrectomy to more closely approximate the response in PD patients undergoing peritoneal dialysis if desired. Increasing the number of rabbits in each group, in future studies would ensure adequate numbers for statistical evaluation of differences between the groups. This would take significant resources. With four rabbits in a group, it takes two people to take PDE, plasma samples and carryout dialysis exchanges in the rabbits on the schedule required. Three people are ideal in the first 2 hours when plasma samples are taken close together. The need for isolation in working with resistant organisms also limits the size of the groups.

This model is ideal for the study of new antibiotic treatments for peritonitis. Antibiotics could be administered i.p., as in this study, i.v. or p.o with complete pharmacokinetic/pharmacodynamic studies of the concentrations achieved in PD and plasma along with studies of efficacy as determined by the eradication of planktonic bacteria in PDE and sessile forms in biofilm along catheter surfaces, the subcutaneous tunnel and peritoneal membrane.

Hotchkiss (4) et al. have criticized current animal models of peritonitis as being chronic in nature, and lacking in closely spaced exchanges for the study of peritoneal washout in treatment of peritonitis. This model could easily be adapted to

fit these needs with any desired number and timing of exchanges. Peritoneal washout could be added as an adjunctive therapy in this rabbit model.
Although much work remains to be done in this area, in the near future, work on MPC and MBEC data may have progressed to the stage where these indices can be used to optimize treatment to ensure prevention of mutant selection as well as the eradication of sessile forms of microorganisms in biofilm. The MPC and MBEC for SYN 1193 against *S. aureus* MU7056 and the proper application of these concepts to in vivo treatment regimens are not known. This model is ideal for the study of the application of the in vitro concept of MBEC. Research in these areas is in its infancy. In the future, we may find that pharmacodynamic parameters relative to these factors, rather than those relative to MIC, correlate best with overall outcome. Choosing the most stringent parameters such as AUC/MPC, t< MPC, peak/MPC, AUC/MBEC, t> MBEC and peak/MBEC may be the ultimate goal in adequately treating these infections, eradicating biofilm and minimizing mutant selection. Most studies to date on enrichment of mutant selection have been done using in vitro studies. Further work needs to be done to elucidate the pharmacodynamic parameters associated with the mutant selection and enrichment in vivo using animal models and human studies. This model could be used to study the in vivo application of these parameters.

Studies by Sepandj et al. (102) with coagulase negative staphylococci have shown that although no resistance to vancomycin was detected using standard MIC testing, 17% resistance was shown on MBEC testing, while no resistance was found on MIC and MBEC testing with a 1:1 combination of vancomycin and rifampin. It would be interesting to study this concept with *S. aureus* to determine if the combination may be more successful in the treatment of the sessile forms of *S. aureus* in biofilm than vancomycin alone. In a study done by Palmer et al., the activity of levofloxacin and vancomycin against MSSA and MRSA were compared in an in vitro fibrin clot model with or without rifampin. Levofloxacin was significantly better than vancomycin or vancomycin with rifampin. Rifampin did not enhance the activity of levofloxacin against either strain but did enhance the activity of vancomycin. (115). It would be interesting to determine if the addition of rifampin enhanced the activity of SYN 1193 against biofilm.

Future approaches to the treatment of peritonitis in peritoneal dialysis may include adjunctive therapy. As more is known about biofilm and it eradication, ancillary measures may be tested with this model as well. Preliminary studies have been done by Carmen et al. demonstrating the enhancement of vancomycin with low frequency ultrasound in the treatment of polyethylene discs covered with *Staphylococcus epidermidis* biofilm implanted in rabbits (116). This group has also demonstrated improved diffusion of gentamicin through biofilms of *P. aeruginosa* and *E. coli.* The rabbit model used in our studies would be ideal for the application of this research to the treatment of biofilm associated infections in peritoneal dialysis. Ultrasound treatments along the subcutaneous catheter tract along with antibiotic treatment may result in enhanced clearing of biofilm. Similar in vitro studies have been done using low intensity electrical field stimulation in addition to antimicrobial agents.

A group of chemicals, furanones, are currently being studied for their effect on inhibition of quorum sensing in the development of biofilm (117).
At the present time, these chemicals are too toxic to be administered to animals, but as this research evolves, the use of substances inhibiting quorum sensing could be used to inhibit or treat infections with biofilm development. Enzymes that break down biofilm are also in the initial stages of research, but represent future opportunities in the treatment of biofilm associated infections (89). It is likely that these enzymes would need to be specific for different bacterial strains due to differences in the exopolysaccharide coating.

In addition to the study of antibiotics, chemicals and ancillary treatments, this model is ideal for the study techniques to prevent the development of biofilm.

Silver-coated PD catheters have been studied by Dasgupta et al. using this rabbit model (118). Silver-catheters catheters showed reduced bacterial growth in comparison to routine Silastic catheters, however, there were some concerns about the release of silver into the tissues.

Conclusions

Rabbit Model

This rabbit model appears to be an excellent animal model for the study of peritonitis in peritoneal dialysis. The pathway of infection follows that of peritonitis following exit site infection in the human with the infection tracking along the subcutaneous the PD catheter, through the Dacron cuff, and into the peritoneal cavity facilitated by dialysis exchanges. All aspects of this infectious process are represented in this model, planktonic bacterial growth in the peritoneal cavity, planktonic or sessile bacterial growth on subcutaneous tissues and peritoneal membrane and sessile bacterial growth in biofilm along the catheter surfaces and Dacron cuff. Integration of pharmacodynamic and efficacy studies can provide a clearer picture as to the tactics to follow in ensuring adequate treatment of planktonic forms and sessile forms of bacteria in biofilm. Efficacy of antibiotic treatment of planktonic organisms can be assessed through cultures of PDE and tissue samples while efficacy of sessile bacterial growth can be assessed through SEM of the subcutaneous tissues, peritoneum and catheter surfaces. The pharmacokinetic and pharmacodynamic indices of the antibiotic can be measured in PDE and plasma to ensure adequate dosing to eradicate infection in the peritoneal cavity as well as along the subcutaneous tunnel and catheter.

This model can be used in the future to study the efficacy of new antimicrobial agents, combinations of antimicrobial agents and adjunctive therapies. Specific studies of biofilm eradication can be done to determine the clinical application of

MBECs and mutant selection prevention using MPCs using this in vivo rabbit model.

SYN 1193

Intraperitoneal administration of SYN 1193 shows promise as an alternative to vancomycin in the treatment peritonitis in patients receiving peritoneal dialysis. After only 4 days of treatment infection in PDE was completely cleared in Rabbits 9 and 11 while rabbit 10 showed a 2.11 $\log_{10}$ or >99% reduction in bacterial growth. Bacterial growth was completely cleared from all tissue/catheter sites in rabbit 9 with only the external catheter showing sessile bacterial growth in biofilm. Although an overall $\log_{10}$ reduction of 3.85 ± 2.27 in bacterial growth was achieved at tissue / catheter sites, Rabbit 10 showed residual bacterial growth at 3 of seven tissue/catheter sampling sites and showed biofilm growth in SEM of the internal catheter but not the external catheter. Rabbit 11 showed residual bacterial growth at 5 of the 7 tissue/catheter sampling sites and biofilm growth on both the internal and external catheter. It is not known if continuing treatment for a complete 14 - 21 day course would clear the remaining sites or if the dose of SYN 1193 must be optimized to ensure adequate concentrations of free SYN 1193 in PDE and plasma as well as optimal pharmacodynamic parameters of free concentrations of SYN 1193 such as *f*peak/MIC ratio and *f*AUC_{24}/MIC ratios. MBEC and MPC are both very important parameters that should be studied with this drug. This rabbit model would be appropriate for in vivo study of both concepts.

SYN 1193 20 mg/kg administered i.p. b.i.d. with the first and last of four dialysis exchanges at 0, 4, 8 and 12 hr each day provided adequate free and total concentrations in PDE for treatment of *S. aureus* MU7056 as well as adequate peak/MIC ratio (free and total) concentrations in PDE for the prevention of resistance. The pharmacodynamic parameters of total peak/MIC ratio (208.06 ± 15.17) and free peak/MIC ratios (41.61 ± 03) in PDE exceed the targets of 8 - 20 correlated with efficacy and prevention of the development of resistance. These original targets had been derived using total concentrations, however, with SYN 1193, protein binding may be an issue due to its 80% protein binding. It is reassuring that the free concentrations in PDE also reach or exceed the targets in PDE. The $AUC/MIC_{(total)}$ ratio (306.48 – 350.78) and $AUC/MIC_{(free)}$ (61.3 – 70.6) achieved in PDE also meet or exceed the target of $AUC/MIC_{(free)}$ ratio of 50-80 for the successful treatment of *S. aureus*. These targets correlate with complete clearing of bacteria in PDE of Rabbits 9 and 10 and reduced bacterial counts of 1.3×10^2 CFU/mL (a >2 $\log_{10}$ reduction in bacterial counts) in PDE of Rabbit 11 and the overall $\log_{10}$ reduction in CFU/mL in PDE of 4.34 ± 1.09 for Rabbits 9 - 11. The $AUC/MIC_{(total)}$ ratio (306.48 – 350.78) achieved in PDE exceeds the AUC/MIC ratio of 100 correlated with prevention of the development of resistance, however the $AUC/MIC_{(free)}$ (61.3 – 70.6) falls short of this target. This target had also been developed using total concentrations but protein binding of SYN 1193 or 80% may make the target of free concentrations a better choice.

Vancomycin

Vancomycin has been used as a positive control in this study. Planktonic and sessile forms of infection in tissue and along catheter surfaces appeared to respond well to only four days of therapy with vancomycin 20mg/kg i.p. q12h in the first and last of 4 dialysis exchanges q.4.h. Infection in PDE completely cleared in rabbits 13A-15A showing a 3.96 ± 0.68 (mean ± SE) $\log_{10}$ (~99.99%) reduction in bacterial counts over the 4 day treatment period. We were unable to evaluate Rabbit 16A due to a blocked catheter.

Tissue and catheter samples in Rabbits 13A and 15A were completely cleared of bacterial growth after four days of treatment with vancomycin. Only one rabbit

(14A) showed bacterial growth at 2 sites, the external catheter (1.1×10^4 CFU/g/mL) and the subcutaneous tunnel (7.7×10^2 CFU/g/mL). Vancomycin treated rabbits showed a mean ± SE $\log_{10}$ reductions in CFU/g/mL for all tissue / catheter samples of 4.98 ± 1.74 compared to controls indicative of a > 99.99% reduction in bacterial growth. Only one of eight samples of internal and external catheter was positive for adherent bacterial growth in biofilm. No statistical difference was found between the $\log_{10}$ reductions in bacterial growth in tissue and catheter sample sites or PDE in the vancomycin and SYN 1193 groups.

Peak plasma concentrations of 19.5 – 33.7 μg/mL fall within the normally accepted range of 20 - 40 μg/mL Trough concentrations were < 3μg/mL in PDE and plasma of all rabbits, however, trough concentrations remained above the MIC for ≥ 10.25 hr of the 12 hr dosing interval. Vancomycin free peak/MIC ratios of 19.5 – 33.7 attained in PDE and plasma exceeded those of 5-6 reported by Knudsen et al. to correlate with treatment efficacy. AUC/MIC ratios of 173 – 303 in plasma and 868 – 1291 in PDE were well in excess of 80 reported by Pavie et al. to correlate with effective therapy (82).

Administration of intraperitoneal vancomycin or SYN 1193 resulted in a >3 $\log_{10}$ reduction in planktonic and sessile forms of methicillin-ciprofloxacin resistant *S. aureus* MU7056. No statistical difference was found between the $\log_{10}$ reductions in bacterial growth in tissue and catheter sample sites or PDE in the vancomycin and SYN 1193 groups although the number of rabbits in each group is small. There is concern that treatment did not completely eradicate the sessile forms of infection along the tissues and catheter surfaces and remaining sessile bacteria in biofilm may serve as a nidus for relapsing or recurrent. This is not surprising since these infections are very resistant to treatment and in practice infections involving biofilm usually cannot be cured without removal the infected material (92). Both drugs looked very promising after only 4 days of treatment. Further studies increasing the duration of the treatment regimen to 14 -21 days would give a clearer picture of the response expected in the clinical setting. Doses of antibiotics could be optimized in future studies. In particular, the free fraction of SYN 1193 would be the optimal target for pharmacodynamic calculations.

Chapter 5

Summary and Conclusions

We were able to further develop an existing rabbit model of exit site infection leading to development of catheter related infection and development of peritonitis for use in the study of the intraperitoneal treatment of peritonitis using a human strain of MRSA. This model is suitable for the study of efficacy of antibiotic treatment taking into consideration microbiologic efficacy of treatment of planktonic and sessile forms of bacteria in biofilm in PDE, and along the subcutaneously implanted catheter. In addition, this model allowed us to integrate pharmacokinetic and pharmacodynamic indices with microbiologic data.

In the development of the rabbit model of exit site infection by Read at al., used in these experiments, it was determined that the inoculation of the exit site of the PD catheter lead to the extensive (>50%) development of biofilm along the surfaces of the catheter and the surrounding subcutaneous tissues. The rate of bacterial biofilm development along the surfaces of the PD catheter, and dissemination of the bacteria into the peritoneal fluid were accelerated by dialysis in this model. It was speculated that eventually biofilm would spread to the peritoneal wall [Read, 1989 #206]. This model has subsequently used by Dasgupta et al. to determine the efficacy of silver-coated PD catheters in the prevention of the development of biofilm (118).

In our studies, this model has proven to be an excellent model for the study of antibiotic treatment of peritonitis in peritoneal dialysis. The model includes an indwelling Silastic Tenckhoff PD catheter of the type used in humans embedded along a subcutaneous tunnel as occurs in PD patients. In humans, catheter-related infection may develop either through touch contamination during manipulations during dialysis exchanges, or as an exit site infection that tracks along the subcutaneous tunnel to the peritoneal cavity leading to the development of peritonitis. In the model used in these studies, infection takes place at the exit site and travels along the subcutaneous tunnel surrounding the catheter, through the Dacron cuff and into the peritoneal cavity. Peritoneal dialysis exchanges impair host defenses and contribute to the development of peritonitis. Peritoneal dialysis exchanges with Dianeal or other fluids such as icodextrin etc. can be performed on a schedule identical to those used in patients if desired or the number of exchanges may be increased to determine the effectiveness of extra exchanges as a mechanism to flush out infection. Antibiotics can be administered by the i.p., i.v., or p.o. routes.

In this model, as in humans, we found that the acute infection in the peritoneal fluid clears quite quickly with appropriate i.p. antibiotic therapy, however adherent bacteria develop into a biofilm along the surfaces of the catheter and persist in a protected environment. Although in vitro methods to study biofilm such as The Modified Robbins Device and The Calgary Biofilm Device have been used, and catheters removed from infected patients have been used to study biofilm, this is the first animal model that incorporates all aspects of the process – catheter implantation along the subcutaneous route into the peritoneal cavity, exit site infection, infection along the catheter and subcutaneous route, peritonitis and regular dialysis exchanges with i.p. administration of antibiotics for the treatment of peritonitis and catheter related infection. Resolution of infection may be studied locally in the peritoneal effluent, the peritoneal membrane or along the subcutaneous tunnel and the indwelling catheter. Pharmacokinetics of drugs used in the treatment of peritonitis can be studied, and pharmacodynamic parameters achieved such as AUC_{24}/MIC ratio, peak/MIC ratio and %t>MIC can be integrated with the microbiologic response. The impact of various treatments on the destruction of biofilm and sessile organisms may also be studied using this model.

This rabbit model is also an excellent model for future studies of the pharmacodynamics of vancomycin since definitive studies are currently lacking and there is no consensus on the optimal pharmacokinetic parameters of vancomycin that correlate with clinical efficacy and peritoneal dialysis complicates the issue. It is also an excellent model for the study of pharmacodynamics within the context of i.p. administration in peritoneal dialysis. Pharmacodynamic parameters such as % t > MIC in PDE may be dramatically altered in this situation and the effects on the reliability of other parameters such as AUC_{24}/MIC ratio and peak/MIC ratios is not known.

In order to do these studies we developed reliable and sensitive assays for SYN 1193 and sparfloxacin. Although we had originally planned to use sparfloxacin as a comparative treatment in this study, development of resistance and photosensitivity made it a less than ideal choice. Problems with the existing sparfloxacin assay requiring direct injection of plasma were overcome with the determination that the extraction of sparfloxacin was pH dependent due to the amphoteric structure of the sparfloxacin molecule.

Using this model, we have studied an investigational fluoroquinolone, SYN 1193 in the treatment of a human strain of ciprofloxacin-resistant, methicillin-resistant *S. aureus*. We studied rabbits treated with vancomycin, as a positive control, and placebo groups for comparison. The results of these studies indicate that SYN 1193 is very promising as an alternative to vancomycin in the treatment of peritonitis and associated catheter-related infections with methicillin-resistant *S. aureus*. Planktonic forms of infection in PDE cleared well with Log_{10} reductions in bacterial growth (CFU/mL) in PDE following treatment with i.p. SYN 1193 of 4.34 ± 1.09 compared to a 3.96 ± 0.68 log_{10} reduction in bacterial growth in the vancomycin group. There was no statistical difference between these groups ($p > 0.05$). Overall log_{10} reduction in bacterial growth in tissue and catheter samples in the SYN 1193 group was 3.85 ± 2.27 compared to 4.98 ± 1.74 in the vancomycin group indicative of a > 99.9% reduction in bacterial growth in both groups after only 4 days of treatment. No statistical difference was found between these groups, however numbers of animals were small. There is concern about residual infection and in particular biofilm remaining along the catheter. However there was a greater than 99.9% reduction in bacterial growth in tissue and catheter samples overall after only 4 days of therapy. Additional studies need to be done to determine if a complete dosing regimen of 14 -21 days will eradicate planktonic and sessile forms of infection at all sites. Additional studies would provide an opportunity to optimize dosing strategies with SYN 1193 taking the free fraction into consideration.

In the future, this model would be ideal to integrate pharmacodynamic and efficacy studies to determine the best approaches to take in ensuring eradication of both planktonic and sessile forms of infection in biofilm and prevention of mutant selection and enhancement. It would also be an excellent model to study the in vivo application of the concepts of MBEC and MPC. In addition this would also be an ideal model for the application of investigate adjunctive treatments of biofilm related infection such as ultrasound, low voltage electrical impulses, specific enzymes to break down the biofilm matrix, chemicals to inhibit quorum sensing in biofilm development or new materials for catheters to prevent the development of biofilm.

References

1. Piraino B, Bailie GR, Bernardini J, et al. Peritoneal dialysis-related infections recommendations: 2005 update. Perit Dial Int 2005;25(2):107-31.

2. Brown EA. An opportune time to develop new strategies against repeat peritonitis in patients on peritoneal dialysis?[comment]. American Journal of Kidney Diseases 2002;39(6):1318-20.

3. Troidle L, Gorban-Brennan N, Kliger A, Finkelstein FO. Continuous peritoneal dialysis-associated peritonitis: a review and current concepts. Seminars in Dialysis 2003;16(6):428-37.

4. Hotchkiss JR, Hermsen ED, Hovde LB, Simonson DA, Rotschafer JC, Crooke PS. Dynamic analysis of peritoneal dialysis associated peritonitis. ASAIO Journal 2004;50(6):568-76.

5. Fried L, Abidi S, Bernardini J, Johnston JR, Piraino B. Hospitalization in peritoneal dialysis patients. Am J Kidney Dis 1999;33(5):927-33.

6. Fried L. Mortality in peritoneal dialysis patients. Asaio J 1999;45(6):526-30.

7. Johnson CC, Baldessarre J, Levison ME. Peritonitis: update on pathophysiology, clinical manifestations, and management. Clin Infect Dis 1997;24(6):1035-45; quiz 46-7.

8. Zelenitsky S, Barns L, Findlay I, et al. Analysis of microbiological trends in peritoneal dialysis-related peritonitis from 1991 to 1998. Am J Kidney Dis 2000;36(5):1009-13.

9. Dasgupta MK. Biofilms and infection in dialysis patients. Semin Dial 2002;15(5):338-46.

10. Keane F, Everett D, Fine R, et al. CAPD related peritonits management and antibiotic therapy recommendations. Travenol Peritonitis Management Advisory Committee. Periotoneal Dialysis Bulletin 1987;7:55 - 62.

11. Keane F, Everett D, Fine R, et al. CAPD related peritonitis management and antibiotic therapy recommendations: an update. Peritoneal Dialysis International 1989;9:247 - 56.

12. Tomasz A. Multiple-antibiotic-resistant pathogenic bacteria. A report on the Rockefeller University Workshop. N Engl J Med 1994;330(17):1247-51.

13. Anonymous. Recommendations for Preventing the Spread of Vancomycin Resistance. Recommendations of the Hospital Infection Control Practices Advisory Committee. Morbidity and Mortality Weekly 1995; 44(RR-12):1 - 3.

14. Smith TL, Pearson ML, Wilcox KR, et al. Emergence of vancomycin resistance in Staphylococcus aureus. Glycopeptide-Intermediate Staphylococcus aureus Working Group. N Engl J Med 1999;340(7):493-501.

15. Fridkin SK, Hageman J, McDougal LK, et al. Epidemiological and microbiological characterization of infections caused by Staphylococcus aureus with reduced susceptibility to vancomycin, United States, 1997-2001. Clinical Infectious Diseases 2003;36(4):429-39.

16. Anonymous. Staphylococcus aureus Resistant to Vancomycin - United States 2002. Morbidity and Mortality Weekly 2002;51(26):565 - 7.

17. Anonymous. Public Health Dispatch: Vancomycin-Resistant Staphylococcus aureus - Pennsylvania, 2002. Morbidity and Mortality Weekly 2002;51(40):902-3.

18. Akpabie A, Naga H, Giraud K, Al Rahiss R, Nadai S. [Resistance to linezolid in Staphylococcus aureus before its release]. Pathol Biol (Paris) 2004;52(8):493-6.

19. Brauers J, Kresken M, Hafner D, Shah PM. Surveillance of linezolid resistance in Germany, 2001-2002. Clin Microbiol Infect 2005;11(1):39-46.

20. Meka VG, Gold HS. Antimicrobial resistance to linezolid. Clin Infect Dis 2004;39(7):1010-5.

21. Golper TA, Schulman G, D'Agata EM. Indications for vancomycin in dialysis patients. Semin Dial 2000;13(6):389-92.

22. Wenzel RP. The antibiotic pipeline--challenges, costs, and values. N Engl J Med 2004;351(6):523-6.

23. Conly J. Where are all the new antibiotics? The new antibiotic paradox. The Canadian Journal of Infectious Diseases and Medical Microbioloby 2005;16(3):159 - 60.

24. SYNPHAR. Antibacterial Activities SYN 1193 against Gram-positive organisms. 1997.

25. Dasgupta MK, Shishido H, Salama S, Singh R, Larabie M, Micetich RG. The effects of macrolide and quinolone antibiotics in methicillin-resistant Staphylococcus aureus biofilm growth. Adv Perit Dial 1997;13:214-7.

26. Kitzis MD, Goldstein FW, Ishida N, Acar JF. SYN987, SYN1193, and SYN1253, new quinolones highly active against gram-positive cocci. Journal of Antimicrobial Chemotherapy 1995;36(1):209-13.

27. O'Donnell JA, Gelone SP. The newer fluoroquinolones. Infectious Disease Clinics of North America 2004;18(3):691-716.

28. Levison ME. Pharmacodynamics of antimicrobial drugs. Infect Dis Clin North Am 2004;18(3):451-65, vii.

29. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clinical Infectious Diseases 1998;26(1):1-10; quiz 1-2.

30. Craig WA. Basic pharmacodynamics of antibacterials with clinical applications to the use of beta-lactams, glycopeptides, and linezolid. Infect Dis Clin North Am 2003;17(3):479-501.

31. Craig WA. Proof of concept: performance testing in models. Clinical Microbiology & Infection 2004;10 Suppl 2:12-7.

32. Ambrose PG, Bhavnani SM, Owens RC, Jr. Clinical pharmacodynamics of quinolones. Infectious Disease Clinics of North America 2003;17(3):529-43.

33. Andes D, Anon J, Jacobs MR, Craig WA. Application of pharmacokinetics and pharmacodynamics to antimicrobial therapy of respiratory tract infections. Clinics in Laboratory Medicine 2004;24(2):477-502.

34. Leggett JE, Ebert S, Fantin B, Craig WA. Comparative dose-effect relations at several dosing intervals for beta-lactam, aminoglycoside and quinolone antibiotics against gram-negative bacilli in murine thigh-infection and pneumonitis models. Scandinavian Journal of Infectious Diseases Supplement 1990;74:179-84.

35. Zhanel GG, Noreddin AM. Pharmacokinetics and pharmacodynamics of the new fluoroquinolones: focus on respiratory infections. Current Opinion in Pharmacology 2001;1(5):459-63.

36. Peloquin CA, Cumbo TJ, Nix DE, Sands MF, Schentag JJ. Evaluation of intravenous ciprofloxacin in patients with nosocomial lower respiratory tract infections. Impact of plasma concentrations, organism, minimum inhibitory

concentration, and clinical condition on bacterial eradication. Arch Intern Med 1989;149(10):2269-73.

37. Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, Schentag JJ. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. Antimicrobial Agents & Chemotherapy 1993;37(5):1073-81.

38. Dudley MN, Blaser J, Gilbert D, Mayer KH, Zinner SH. Combination therapy with ciprofloxacin plus azlocillin against Pseudomonas aeruginosa: effect of simultaneous versus staggered administration in an in vitro model of infection. J Infect Dis 1991;164(3):499-506.

39. Drusano GL, Johnson DE, Rosen M, Standiford HC. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of Pseudomonas sepsis. Antimicrobial Agents & Chemotherapy 1993;37(3):483-90.

40. Preston SL, Drusano GL, Berman AL, et al. Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials.[see comment]. JAMA 1998;279(2):125-9.

41. Lister PD. Impact of AUC/MIC ratios on the pharmacodynamics of the des-F(6) quinolone garenoxacin (BMS-284756) is similar to other fluoroquinolones. Journal of Antimicrobial Chemotherapy 2003;51(1):199-202.

42. Turnidge J. Pharmacokinetics and pharmacodynamics of fluoroquinolones. Drugs 1999;58 Suppl 2:29-36.

43. Lister PD, Sanders CC. Pharmacodynamics of levofloxacin and ciprofloxacin against Streptococcus pneumoniae. Journal of Antimicrobial Chemotherapy 1999;43(1):79-86.

44. Lacy MK, Lu W, Xu X, et al. Pharmacodynamic comparisons of levofloxacin, ciprofloxacin, and ampicillin against Streptococcus pneumoniae in an in vitro model of infection. Antimicrobial Agents & Chemotherapy 1999;43(3):672-7.

45. Hershberger E, Coyle EA, Kaatz GW, Zervos MJ, Rybak MJ. Comparison of a rabbit model of bacterial endocarditis and an in vitro infection model with simulated endocardial vegetations. Antimicrobial Agents & Chemotherapy 2000;44(7):1921-4.

46. Lister PD. Pharmacodynamics of gatifloxacin against Streptococcus pneumoniae in an in vitro pharmacokinetic model: impact of area under the

curve/MIC ratios on eradication. Antimicrob Agents Chemother 2002;46(1):69-74.

47. Jumbe N, Louie A, Liu, W., Bachawat, R. Freeman C., Ma, J. Tam, V., Deziel, M., Miller, M., Drusano, G. Pharmacokinetics of Levofloxacin with Differnet Oganisms: One Size Does Not Fit All. In: Interscience Conference of Antimicrobial Chemotherapy American Society for Microbiology; 2000; Toronto, Ontario, Canada; 2000.

48. Bergogne-Berezin E. Clinical role of protein binding of quinolones. Clinical Pharmacokinetics 2002;41(10):741-50.

49. SYNPHAR. Protein Binding SYN 1193. In. Edmonton, Alberta.; 2005.

50. Craig W, Andes, DA. Correlation of the maginitude of AUC24/MIIC for 6 fluoroquinolones against Sreptococcus pneumoniae with survivial and bactericidal activity in an animal model. In: 40th Inbterscience Conference of Antimicrobial Chemotherapy; 2000; Toronto, Canada; 2000.

51. Andes D, Craig WA. Pharmacodynamics of the new fluoroquinolone gatifloxacin in murine thigh and lung infection models. Antimicrobial Agents & Chemotherapy 2002;46(6):1665-70.

52. Andes D, Craig WA. Pharmacodynamics of the new des-f(6)-quinolone garenoxacin in a murine thigh infection model. Antimicrobial Agents & Chemotherapy 2003;47(12):3935-41.

53. Sanders CC. Mechanisms responsible for cross-resistance and dichotomous resistance among the quinolones. Clin Infect Dis 2001;32 Suppl 1:S1-8.

54. Drlica K, Malik M. Fluoroquinolones: action and resistance. Curr Top Med Chem 2003;3(3):249-82.

55. Thomas JK, Forrest A, Bhavnani SM, et al. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. Antimicrob Agents Chemother 1998;42(3):521-7.

56. Craig WA. Does the dose matter? Clinical Infectious Diseases 2001;33 Suppl 3:S233-7.

57. Jumbe N, Louie A, Leary R, et al. Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy. J Clin Invest 2003;112(2):275-85.

58. Tam VH LA, Deziel MR, Liu W, Frasela DM, Miller MH, et al. Pharmacokinetics of BMS-284756 in counter-selecting resistance in a hollow-fibre system. Abstracts of the 41st Interscience Conference of Antimicrobial Chemotherapy 2001.

59. Tam VH, Louie A, Deziel M.R., Liu, W., Grasela, D.M., Miller, M.H. et al. Pharmacokinetics of BMS 284756 in counter-selecting resistance in a hollow-fibre system. In: Interscience Conference of Antimicrobial Chemotherapy, American Society of Microbiology; 2001; Chigago; 2001.

60. Firsov AA, Vostrov SN, Lubenko IY, Arzamastsev AP, Portnoy YA, Zinner SH. ABT492 and levofloxacin: comparison of their pharmacodynamics and their abilities to prevent the selection of resistant Staphylococcus aureus in an in vitro dynamic model. Journal of Antimicrobial Chemotherapy 2004;54(1):178-86.

61. Drlica K. The mutant selection window and antimicrobial resistance. J Antimicrob Chemother 2003;52(1):11-7.

62. Blondeau JM, Hansen G, Metzler K, Hedlin P. The role of PK/PD parameters to avoid selection and increase of resistance: mutant prevention concentration. J Chemother 2004;16 Suppl 3:1-19.

63. Dong Y, Zhao X, Domagala J, Drlica K. Effect of fluoroquinolone concentration on selection of resistant mutants of Mycobacterium bovis BCG and Staphylococcus aureus. Antimicrobial Agents & Chemotherapy 1999;43(7):1756-8.

64. Zhao X, Karl D. Restricting the Selection of Antibiotic-Resistanct Mutants:A General Strategy Derived from Fluoroquinolone Studies. CLinical Infecitous Diseases 2001;33(Suppl 3):S147 - 56.

65. Allen GP, Kaatz GW, Rybak MJ. In vitro activities of mutant prevention concentration-targeted concentrations of fluoroquinolones against Staphylococcus aureus in a pharmacodynamic model. Int J Antimicrob Agents 2004;24(2):150-60.

66. Campion JJ, Chung P, McNamara PJ, Titlow WB, Evans ME. Pharmacodynamic modeling of the evolution of levofloxacin resistance in Staphylococcus aureus. Antimicrob Agents Chemother 2005;49(6):2189-99.

67. Campion JJ, McNamara PJ, Evans ME. Evolution of ciprofloxacin-resistant Staphylococcus aureus in in vitro pharmacokinetic environments. Antimicrobial Agents & Chemotherapy 2004;48(12):4733-44.

68. Firsov AA, Vostrov SN, Lubenko IY, Zinner SH, Portnoy YA. Concentration-dependent changes in the susceptibility and killing of Staphylococcus aureus in an in vitro dynamic model that simulates normal and impaired gatifloxacin elimination. Int J Antimicrob Agents 2004;23(1):60-6.

69. Moellering RC, Jr., Krogstad DJ, Greenblatt DJ. Vancomycin therapy in patients with impaired renal function: a nomogram for dosage. Ann Intern Med 1981;94(3):343-6.

70. Matzke GR, McGory RW, Halstenson CE, Keane WF. Pharmacokinetics of vancomycin in patients with various degrees of renal function. Antimicrob Agents Chemother 1984;25(4):433-7.

71. Lake KD, Peterson CD. Evaluation of a method for initiating vancomycin therapy: experience in 205 patients. Pharmacotherapy 1988;8(5):284-6.

72. Golper TA, Noonan HM, Elzinga L, et al. Vancomycin pharmacokinetics, renal handling, and nonrenal clearances in normal human subjects. Clin Pharmacol Ther 1988;43(5):565-70.

73. Moellering RC, Jr. Monitoring serum vancomycin levels: climbing the mountain because it is there?[erratum appears in Clin Infect Dis 1994 Aug;19(2):379]. Clinical Infectious Diseases 1994;18(4):544-6.

74. Cantu TG, Yamanaka-Yuen NA, Lietman PS. Serum vancomycin concentrations: reappraisal of their clinical value. Clin Infect Dis 1994;18(4):533-43.

75. Shafran SD. The serum vancomycin assay: a test of historic interest. Canadian Journal of Infectious Diseases 1995;6(2):67-8.

76. Rodvold KA, Blum RA, Fischer JH, et al. Vancomycin pharmacokinetics in patients with various degrees of renal function. Antimicrob Agents Chemother 1988;32(6):848-52.

77. Freeman CD, Quintiliani R, Nightingale CH. Vancomycin therapeutic drug monitoring: is it necessary? Annals of Pharmacotherapy 1993;27(5):594-8.

78. Ackerman BH. Evaluation of three methods for determining initial vancomycin doses. Dicp 1989;23(2):123-8.

79. Mulhern JG, Braden GL, O'Shea MH, Madden RL, Lipkowitz GS, Germain MJ. Trough serum vancomycin levels predict the relapse of gram-positive peritonitis in peritoneal dialysis patients. Am J Kidney Dis 1995;25(4):611-5.

80. Zimmerman AE, Karona, B.G., Plaisance, K.I. Association of Vancomyin Serum Concentrations With Outcome in Patients with Gram-Positive Bactermenia. Pharmacotherapy 1995;15(1):85-91.

81. Duffull SB, Begg EJ, Chambers ST, Barclay ML. Efficacies of different vancomycin dosing regimens against Staphylococcus aureus determined with a dynamic in vitro model. Antimicrob Agents Chemother 1994; 38(10):2480-2.

82. Pavie J, Lefort A, Ploy MC, et al. Influence of reduced susceptibility to glycopeptides on activities of vancomycin and teicoplanin against Staphylococcus aureus in experimental endocarditis. Antimicrob Agents Chemother 2003;47(6):2018-21.

83. Moise PA FA, Bhavmani SM, Birmkingham MC, Schentag JJ. Area under the inhibitory curve and a pneumonia scoring system for predicting outcomes of vancomycin theroapy for respiratory invfections by Staphylococcus aureus. American Journal of Health Systems Phamacists 2000;57 (Suppl 2):4-9.

84. Moise-Broder PA, Forrest A, Birmingham MC, Schentag JJ. Pharmacodynamics of vancomycin and other antimicrobials in patients with Staphylococcus aureus lower respiratory tract infections. Clinical Pharmacokinetics 2004;43(13):925-42.

85. Knudsen JD, Fuursted K, Espersen F, Frimodt-Moller N. Activities of vancomycin and teicoplanin against penicillin-resistant pneumococci in vitro and in vivo and correlation to pharmacokinetic parameters in the mouse peritonitis model. Antimicrob Agents Chemother 1997;41(9):1910-5.

86. Costerton JW, Stewart PS. Battling biofilms. Scientific American 2001;285(1):74-81.

87. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical Microbiology Reviews 2002;15(2):167-93.

88. Whiteley MB, M. Bumgarner, R. Parsek, M. Teltzel, G. Lory, S. Greenberg, E. Gene expression in Pseudomonas aeuginas biofilms. Nature 2001(September 2001).

89. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358(9276):135-8.

90. Yarwood JM, Schlievert PM. Quorum sensing in Staphylococcus infections. Journal of Clinical Investigation 2003;112(11):1620-5.

91. Costerton JW. Introduction to biofilm. Int J Antimicrob Agents 1999;11(3-4):217-21; discussion 37-9.

92. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284(5418):1318-22.

93. Davies D. Understanding biofilm resistance to antibacterial agents. Nature Reviews Drug Discovery 2003;2(2):114-22.

94. Stone G, Wood P, Dixon L, Keyhan M, Matin A. Tetracycline rapidly reaches all the constituent cells of uropathogenic Escherichia coli biofilms. Antimicrob Agents Chemother 2002;46(8):2458-61.

95. Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. Antimicrob Agents Chemother 2000;44(7):1818-24.

96. Stewart PS. Diffusion in biofilms. Journal of Bacteriology 2003;185(5):1485-91.

97. Anwar H, Dasgupta M, Lam K, Costerton JW. Tobramycin resistance of mucoid Pseudomonas aeruginosa biofilm grown under iron limitation. J Antimicrob Chemother 1989;24(5):647-55.

98. Anwar H, van Biesen T, Dasgupta M, Lam K, Costerton JW. Interaction of biofilm bacteria with antibiotics in a novel in vitro chemostat system. Antimicrob Agents Chemother 1989;33(10):1824-6.

99. Sepandj F, Ceri H, Gibb A, Read R, Olson M. Minimum inhibitory concentration (MIC) versus minimum biofilm eliminating concentration (MBEC) in evaluation of antibiotic sensitivity of gram-negative bacilli causing peritonitis. Peritoneal Dialysis International 2004;24(1):65-7.

100. Ceri H, Olson M, Morck D, et al. The MBEC Assay System: multiple equivalent biofilms for antibiotic and biocide susceptibility testing. Methods in Enzymology 2001;337:377-85.

101. Olson ME, Ceri H, Morck DW, Buret AG, Read RR. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Canadian Journal of Veterinary Research 2002;66(2):86-92.

102. Sepandj F, Ceri H, Gibb AP, Read RR, Olson M. Biofilm infections in peritoneal dialysis-related peritonitis: comparison of standard MIC and

MBEC in evaluation of antibiotic sensitivity of coagulase-negative staphylococci. Peritoneal Dialysis International 2003;23(1):77-9.

103. Read RR, Eberwein P, Dasgupta MK, et al. Peritonitis in peritoneal dialysis: bacterial colonization by biofilm spread along the catheter surface. Kidney Int 1989;35(2):614-21.

104. O'Donnell JA, Gelone SP. Fluoroquinolones. Infectious Disease Clinics of North America;14(2):489-513.

105. Lyon D, Cheung S, Chan C, Cheng A. Rapid HPLC asay of clinafloxacin, fleroxacin, levofloxacin sparfloxacin and tosufloxacin. J Antimicrob Chemother 1994;34:446-8.

106. Diem K, Lentner C. Geigy Scientific Tables. 7th ed. Basa Switzerland: Ciba Geigy Ltd; 1973.

107. Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. Journal of Infectious Diseases 1988; 158(4):831-47.

108. Schentag JJ, Strenkoski-Nix LC, Nix DE, Forrest A. Pharmacodynamic interactions of antibiotics alone and in combination. Clin Infect Dis 1998;27(1):40-6.

109. Schentag JJ. Antimicrobial action and pharmacokinetics/pharmacodynamics: the use of AUIC to improve efficacy and avoid resistance. J Chemother 1999;11(6):426-39.

110. McDermid KP, Morck DW, Olson ME, et al. A porcine model of Staphylococcus epidermidis catheter-associated infection. J Infect Dis 1993;168(4):897-903.

111. Andes D, Craig WA. Animal model pharmacokinetics and pharmacodynamics: a critical review. International Journal of Antimicrobial Agents 2002;19(4):261-8.

112. Hanberger H, Nilsson LE, Maller R, Isaksson B. Pharmacodynamics of daptomycin and vancomycin on Enterococcus faecalis and Staphylococcus aureus demonstrated by studies of initial killing and postantibiotic effect and influence of Ca2+ and albumin on these drugs. Antimicrobial Agents & Chemotherapy 1991;35(9):1710-6.

113. Bailey EM, Rybak MJ, Kaatz GW. Comparative effect of protein binding on the killing activities of teicoplanin and vancomycin. Antimicrobial Agents & Chemotherapy 1991;35(6):1089-92.

114. Bennett WM, Aronoff GR, Golper TA, Morrison G, Brater DC, Singer I. Drug Prescribing in Renal Failure: American College of Physicians; 1994.

115. Palmer SM, Rybak MJ. Pharmacodynamics of once- or twice-daily levofloxacin versus vancomycin, with or without rifampin, against Staphylococcus aureus in an in vitro model with infected platelet-fibrin clots. Antimicrob Agents Chemother 1996;40(3):701-5.

116. Carmen JC, Roeder BL, Nelson JL, et al. Ultrasonically enhanced vancomycin activity against Staphylococcus epidermidis biofilms in vivo. J Biomater Appl 2004;18(4):237-45.

117. Hentzer M, Eberl L, Nielsen J, Givskov M. Quorum sensing: a novel target for the treatment of biofilm infections. Biodrugs 2003;17(4):241-50.

118. Dasgupta MK. Silver-coated catheters in peritoneal dialysis. Perit Dial Int 1997;17 Suppl 2:S142-5.